

A COMPARISON OF THE GLYCEMIC INDEX OF SORGHUM AND OTHER  
COMMONLY CONSUMED GRAINS

by

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## Abstract

Little *in vivo* research on glycemic index (GI) values or the digestive impact of sorghum based food products currently exists. Because sorghum is a gluten-free grain, its utilization in the United States is growing, especially in baking applications. Information on how sorghum affects blood sugar levels would be beneficial as new products emerge because glycemic effect has an impact on human health and in controlling diabetes. The objective of this study was to determine the GI of a sorghum muffin, and compare this value to the GI of muffins made from commonly consumed grains in the United States. The effects of particle size and damaged starch on GI were also studied. GI values were determined for muffins made from white sorghum, corn, brown rice, whole wheat, and all-purpose flours. All muffin formulations were composed of flour, water, baking powder and salt. To determine the GI, weighed portions of muffin containing 20g of available carbohydrates were eaten on separate occasions by eight healthy volunteers (ages 18-40) after an overnight fast (10 hours). Each muffin was administered twice. Two capillary blood samples were taken at 0 (fasting), 30, 45, 60, 90 and 120 minutes after consumption and averaged. Blood glucose curves were constructed from mean blood glucose values. The GI was calculated by dividing the incremental area under the curve for the test food (muffin) by that for the standard (20g dextrose drink) and multiplying by 100. The GI for the muffins was calculated as the mean from the respective average GIs of the 8 volunteers. The data indicated that sorghum flour milled at particle size < 400  $\mu\text{m}$  resulted in the lowest GI of  $32 \pm 16.8$ . These findings should assist in development of lower GI sorghum foods.

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# Chapter 1 - Review of Literature

## Sorghum

### *Plant Structure*

*Sorghum bicolor* L. *Moenchi* is an ancient, cereal grain that comes from the grass family Poaceae. Each plant consists of roots, a stalk, leaves of various shape and sizes, and a panicle, or seed head as seen in Figure 1.1 (U.S. Grains Council 2008). The kernels, or caryopses of the panicle may vary in size and shape, but are usually spherical to tear drop shaped and range from 4-8 mm in diameter. A depiction of the caryopses is seen in Figure 1.1 (Dried Botanical ID 2010). Glumes cover the caryopses in an intact plant. Color of the caryopses also vary between several shades of white, yellow, bronze, brown, black, pink, purple and reddish. Varieties most commonly grown in the United States are whites and browns.

Similar to most cereal grains, the average caryopsis consists of three main components: 6% pericarp, 10% germ, and 84% endosperm (FAO 1995). The thick pericarp is then further divided into three layers: epicarp, mesocarp and endocarp. The testa layer resides under the pericarp and may be absent, partially present, or fully present depending on genetic control (Delcour and Hosenev 2010, FAO 1995). The aleurone layer is a single layer of cells surrounding the endosperm (FAO 1995). The endosperm contains both vitreous (corneous) and opaque (floury) endosperm (Delcour and Hosenev 2010). The germ contains the embryonic axis and the scutellum (FAO 1995).





**Figure 1.1 Sorghum plant structure diagram (left). Source: U.S. Grains Council 2008 White sorghum caryopses (right). Source: Dried Botanical ID 2010**

### *Composition*

Sorghum grain is rich in macronutrients, micronutrients and antioxidants. Aside from the presence of resistant starch and antioxidants, the composition of sorghum is strikingly similar to that of maize (Smith and Frederickson 2000). Sorghum's composition varies on account of variety, and environmental conditions. The main constituent of sorghum is starch followed by protein, non-starch polysaccharides and fat.

Most starch in sorghum resides in the endosperm, but a small degree lies in the mesocarp layer of the pericarp. The two starch polymers present are highly branched amylopectin at 70-80%, and linear amylose at 20-30% (Dicko and others 2006). There are a few varieties of waxy sorghum that will contain almost entirely amylopectin (Delcour and Hoseney 2010). Non-starch polysaccharides consist mainly of arabinoxylans and various  $\beta$ -glucans, and are concentrated in the pericarp; however they can also be found minimally in the endosperm cell walls (Verbruggen and others 1993). Lignin polymers are also found in the endosperm cell walls (Hatfield 1999). Niba

and Hoffman (2003) reported that sorghum contains approximately 6.5% resistant starch.

Both the germ and aleurone layer of sorghum are rich in lipids, proteins, enzymes, vitamins and minerals. Sorghum contains about 7-15% protein from albumins, globulins, kafirins (prolamin), crosslinked kafirins and glutelins. The most dominant protein in sorghum is kafirins. Lysine is the limiting amino acid, although there are some high lysine varieties. Crude fat content at about 3% stems mainly from the germ, and is predominantly composed of polyunsaturated fatty acids. In general, sorghum is a good source for vitamin B and vitamin E. Yellow pigmented sorghum is known to be a source of  $\beta$ -carotene which is converted to vitamin A in the body (FAO 1995). Sorghum is also said to be a good source of more than 20 minerals (BSTID-NRC 1996).

A unique attribute of sorghum is its phytochemical content. Condensed tannins are located in the testa of sorghum whose presence is genetically controlled (Delcour and Hosoney 2010). Tannins are classified as type I (no tannins), type II (in testa) or type III (in testa and pericarp). Of the sorghum grown in the United States, 99% is of type I due to breeding efforts since tannins reduce feed value (Smith and Frederiksen 2000). Tannins act as a protective mechanism to the plant, and exhibit antioxidant properties to humans. Some tannins found in sorghum are the same as those found in foods commonly known as super fruits for their antioxidant levels such as pomegranates, blueberries and cranberries (Yang and Chien 2000; Gu and others 2002). Pigmented sorghums, especially black sorghums, also possess anthocyanins (Awika and Rooney 2004). Anthocyanin, a flavonoid triggers genetic signaling in promoting human health and disease prevention (Zafra-Stone and others 2007).

### *Production*

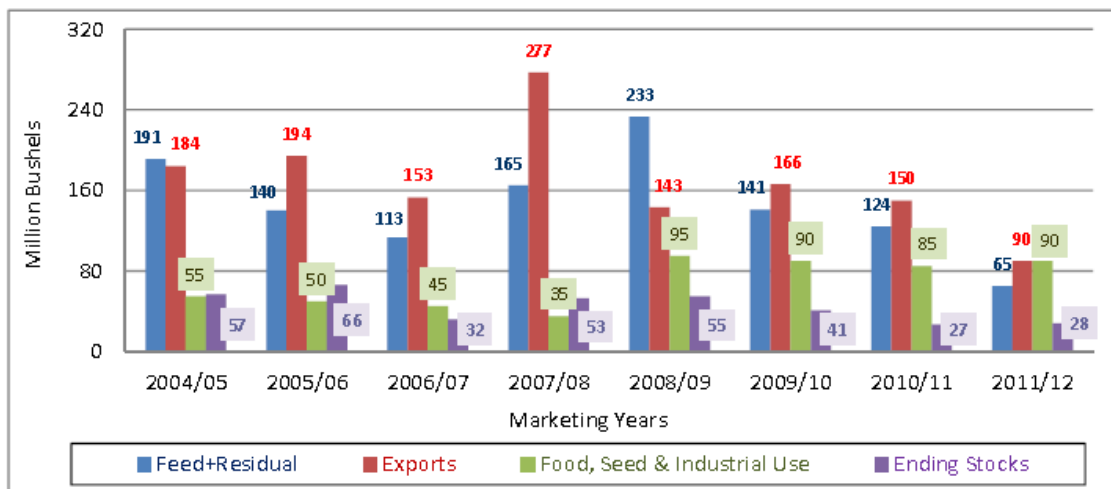
In 2010, world production of sorghum was about 59.5 million metric tons. The top three producers worldwide in 2010 were Nigeria, United States and India. The United States harvested approximately 9.7 million acres in the 2009-2010 season. The leading producers of sorghum in the United States are Kansas, Texas, Nebraska, Oklahoma and Missouri. Leading exporters are United States, Australia and Argentina. The leading cereal grain produced in Africa is sorghum (U.S. Grains Council 2010). It is possible for sorghum to be successfully grown on a global basis due to the crop being able to withstand harsh environments subject to drought, and it requires minimal maintenance overall in terms of fertilizer and irrigation. Different cultivars are produced globally depending on local climate (FAO 1995).

### *Utilization*

Sorghum's primary application varies around the world, but globally speaking sorghum's primary usage is for human food (United Sorghum Checkoff Program 2012). Sorghum is a staple to the countries of Africa and Asia who consume 95% of sorghum worldwide (FAO 1995). In Africa sorghum is usually consumed as porridge or couscous (U.S. Grains Council 2010). Malted sorghum is also used as a weaning food in Africa. India typically boils sorghum like rice or creates unleavened bread (FAO 1995). Japan has recently been innovative in creating snack products from sorghum (US Grains Council 2010). Other common food applications across the globe include malted beverages, syrup, flaked cereal and tortillas (FAO 1995).

The United States, South America, Mexico and Australia use sorghum primarily for animal feed. Other uses include pet food and ethanol production (U.S. Grains

Council 2010). There are also several industrial uses for sorghum such as building materials, fencing, floral arrangements and brooms (United Sorghum Checkoff Program 2012). A breakdown of the United States sorghum usage in recent years can be seen in Figure 1.2 (USDA 2011). Flour from white sorghum is being used for its mild flavor in exploring many applications such as cookies, cakes, brownies, bread, pizza dough, pastas, pancakes, waffles and more (United Sorghum Checkoff Program 2012). Because sorghum is gluten free and has a high gelatinization temperature, some functionality challenges are faced when using sorghum in many applications. Addition of specialty starches and hydrocolloids have been shown to successfully aid in these problems (FAO 1995).



**Figure 1.2 Trends in U.S. grain sorghum use & ending stocks during 2004/2005 thru 2011/2012.**

**Source: USDA WADSE Report 2011**

### *Gluten Free Market*

As celiac disease becomes more prevalent through awareness and diagnosis, the gluten free market is rapidly expanding. The gluten free market is currently the

fastest growing segment of the global food allergy and food intolerance products market (CompaniesandMarkets.com 2011). Packaged Facts (2011) reported that the gluten free market reached an estimated \$2.6 billion in sales for 2010, and is expected to reach \$6 billion in sales by 2015 (Food Product Design 2011). This specialty niche has officially become a mainstream market in just a few years (Packaged Facts 2011). This growth is based on consumers who eat gluten free out of medical necessity, the vast amount of new products available on the shelves and bandwagon consumers (Food Product Design 2011). Interestingly enough, the consumer base for gluten free diets are dominated by non-celiacs due to beliefs that this diet is healthier than others, and that it can be used to treat a number of ailments (CompaniesandMarkets.com 2011). The United States is reported to have the largest gluten free market globally (Packaged Facts 2011).

As a result of this movement, the FDA reopened the proposed rule on gluten free labeling of products in 2010 (Food Product Design 2011). Also in 2010, the United States witnessed large food manufacturers reformulate or re-label products in effort to enter the gluten free market. Such companies are Kellogg's, Betty Crocker, Frito Lay, General Mills, Post and Snyder's of Hanover. Supermarkets like Whole Foods and HyVee are tailoring there stores to be more gluten free friendly by offering gluten free shopping lists, and dedicating specific areas of their stores to gluten free products (Food Processing 2012).

#### *Celiac Disease*

Celiac disease, also known as Celiac Sprue or gluten sensitive enteropathy is an autoimmune disease (CSA 2012). The autoimmune reaction is elicited by gluten protein.

After consumption of gluten, damage and subsequent malfunction to intestinal villi of the small intestine occurs. Intestinal villi are the sites where most nutrient absorption takes place in the digestive system, therefore if the disease goes untreated, malnourishment, other illness and even mortality may result. Celiac disease can occur at any age and at any point in one's life (PubMed 2010). The exact trigger of this disease is unknown, but most people who are diagnosed have been genetically predisposed (Mayo Clinic 2011).

Symptoms of celiac disease vary on an individual basis, and most commonly are associated with the gastrointestinal tract. Common daily symptoms include: abdominal pain, constipation, decreased appetite, diarrhea, lactose intolerance, nausea and vomiting, bloody, fatty, or foul smelling stools, and weight loss. Once the disease has progressed and damage to the intestinal villi is present, other symptoms associated with malnutrition will ensue. These symptoms include: bruising, depression, anxiety, fatigue, hair loss, itchy skin, missed menstrual cycles, mouth ulcers, muscle cramps, joint pain, nosebleeds and seizures.

There is currently no cure for celiac disease. Individuals with celiac disease must adhere to a gluten free lifestyle by abstaining from any food, beverage or medication that may contain traces of barley, rye or wheat. Symptoms will subside and damage caused to the intestine is reversible when maintaining a gluten free lifestyle. It may take upwards of 2-3 years for complete healing, but symptoms typically subside quickly (PubMed 2010). Other treatments are the use of vitamins to combat any malnutrition that may have resulted, and anti-inflammatory drugs until symptoms subside (Mayo Clinic 2011).

Celiac disease is present in about 1 in 133 people in the United States alone. Many are believed to still be undiagnosed.

Aside from celiac disease there are two similar conditions. Six percent of the United States population exhibits gluten sensitivity. This causes similar gastrointestinal symptoms after the ingestion of gluten, but is unique from celiac disease in that no damage to the intestinal villi occurs. Many individuals have a food allergy to wheat (CSA 2011). These conditions are in turn shaping consumer perceptions of gluten, and redefining the gluten free market as we know it.

### ***Sorghum's Niche***

Although sorghum has been used for centuries in developing countries as food, its presence in the United States food market is recent. Sorghum is currently being utilized for its gluten free property in the United States market. There is however a void in information on sorghum's metabolic properties. Because sorghum is a carbohydrate based food, it is important to understand its role in digestion. It is known that sorghum is gluten free and has a good nutritional profile, but with additional nutrition based research there is potential for the grain's position in a specialized market to become mainstream.

## **Glycemic Response**

### ***Carbohydrate Classification***

The main function of carbohydrates in the human body is as an energy source. There are many forms of dietary carbohydrate. Monosaccharides are most commonly referred to as simple sugars. Monosaccharides include glucose (or dextrose), fructose and galactose. Disaccharides are a pair of monosaccharides and consist of sucrose, lactose and maltose. These sugars break down into their subsequent monosaccharides in the body during digestion. Oligosaccharides are composed of 2-10 monosaccharides, and are largely unchanged in the digestive tract until they reach the colon where they are subject to fermentation. Polysaccharides contain more than 10 monosaccharide units, and are recognized as starch and fiber. The two types of starch are amylose and amylopectin. Starches are digested and then absorbed primarily as glucose in the body. Fiber is not digested, but rather passes through the digestive tract in which portions may be fermented in the colon. There is also resistant starch which is resistant to metabolic enzymes and tends to act similar to fiber in the gastrointestinal tract (Barasi 2003).

### ***Role and Regulation of Glucose***

For the human body to operate properly and maintain overall health it must remain in a state of homeostasis. One important process in sustaining homeostasis is effective glucose regulation, as glucose is the primary source of fuel for the brain. Digestible carbohydrates travel through the gastrointestinal tract being broken down into simple sugars. These sugars are absorbed in the small intestine where they are sent into the blood stream. The blood will circulate glucose through the body to tissues and organs for utilization of fuel. As this is occurring, the subsequent increase in blood



glucose will also trigger the release of the hormone insulin from the pancreas. Insulin promotes uptake and storage of glucose in the blood within the liver and muscle in the form of glycogen. Insulin's role as a regulatory hormone is to maintain blood glucose within a healthy range of 3.5-8.0 mmol/L and <7.0% HbA1<sub>c</sub> (Barasi 2003).

### *Glycemic Index*

The glycemic index (GI) was conceived in 1981 by David Jenkins at the University of Toronto for the purpose of ranking carbohydrate based foods on how they elevate blood glucose levels. GI is measured by comparing the effect on blood glucose over a two hour period from 50g available carbohydrate of a test food to 50g available carbohydrate of a reference food. This portion (50g) represents the greatest peak in response to food. Responses plateau after this point (Brouns and others 2005). Reference foods can be either white bread or pure glucose. Test foods are then assigned a category based on their 0-100 ranking as seen in Table 1.1 below (AICR 2008).

<b>Category</b>	<b>Glycemic Index</b>
Low	0-55
Intermediate	56-69
High	≥70

**Table 1.1 Glycemic Index Categories**

### *Glycemic Index Methodology*

When conducting GI trials it is standard to use 10 subjects (Brouns and others 2005). Brouns and others (2005) reported that there is greater precision in results as number of subjects increase. Any combination of male or female is acceptable, and

does not seem to alter results. It is recommended that no rigorous exercise, smoking or diet out of the normal occurs on the day prior to testing in efforts of keeping metabolism consistent (Brouns and others 2005).

It is standard practice to use 50g available carbohydrate for both the test food(s) and the reference food. This is calculated by subtracting the total fiber of the food from the total carbohydrate. This method however does not account for a food's resistant starch component, which may contribute to a degree of error (Wolever and others 1991). As some foods exhibit low carbohydrate density, it is acceptable to use 25g test food and reference food amounts when needed. Water should be served along with the test food or reference food, and all should be consumed within the first 10 minutes of testing (Brouns and others 2005).

If testing more than one food in a trial period, test foods should be administered in random order and on separate occasions. Due to day to day variation within subjects, the reference food should be tested two to three times and averaged (Wolever and others 1991). Brouns and others (2005) recommended that foods be randomized in blocks of up to 6 foods with the reference food being tested before and after each block, and that the total test period duration should not exceed four months. All testing should take place during the morning after a 10-14 hour overnight fast. Fasting conditions have been found most stable in terms of intra-individual differences during the morning (Brouns and others 2005). For healthy subjects, capillary finger prick blood samples are to be obtained at the following points: 0 (fasting), 15, 30, 45, 60, 90 and 120 minutes (Wolever and others 1991). For subjects with diabetes, blood should be measured at 0 (fasting), 30, 60, 90, 120, 150 and 180 minutes (Brouns and others 2005).

Blood glucose response curves are generated by blood glucose readings versus time increments as seen below in Figure 1.3. When calculating GI, the incremental area under the blood glucose response curve (iAUC) for the test food is divided by the incremental area under the blood glucose response curve for the reference food, and the result is multiplied by 100. The incremental area under the blood glucose curve is calculated for each blood glucose response curve geometrically by the trapezoid rule, disregarding the area below the baseline. The basic equation is as follows (Wolever and others 1991):

$$iAUC = (A + B + C + D/2)t + \frac{D^2 t}{2} (D + |\epsilon|)$$

Although there does not seem to be a standard rule for dealing with outliers, Wolever and others (1991) reported that if a test result is greater than two standard deviations from the mean, further testing can be done to replace the outlier given these tests prove more representative.

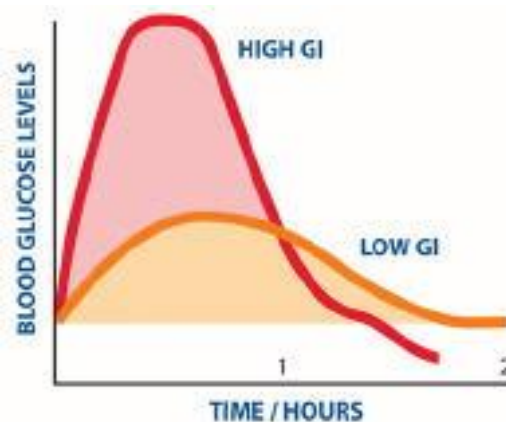


Figure 1.3 Blood glucose response curves. Source: The University of Sydney 2012

### *Glycemic Index Utilization*

The principal purpose of the glycemic index is a tool for food choice. The Glycemic Index Foundation of Australia launched a labeling system in 2002 known as the Glycemic Index Symbol Program. A food with the trademarked 'Low GI Symbol' (Figure 1.4) on the package must be a carbohydrate based food, low in total fat, saturated fat, sodium, calories and be a good source of fiber. The symbol ensures an overall healthy food choice (Glycemic Index Symbol 2012). The University of Sydney claims the GI is a tool for healthy eating for all people (University of Sydney 2012). The Glycemic Index Foundation of South Africa has also endorsed a symbol for low GI foods as seen below in Figure 1.4. Both countries have active databases available for the public with categorized foods. Super markets in the United Kingdom have incorporated GI information and claims onto their packaging as well (Mitchell 2008).

The GI has also been considered in the food industry when developing new products. Kraft Foods Inc. currently has a patent pending *in vitro* method of quickly predicting the GI of foods. Kraft intends on using this as a screening tool for products, but not for labeling purposes (Nutraceuticals World 2010). Another company who has done substantial work on GI is Kellogg's company. Kellogg's Australia has a page on their website dedicated to GI information and classification of their products (Kellogg's 2010).



**Figure 1.4** Low Glycemic Index Symbol of Australia (left). Source: Glycemic Index Symbol 2012  
Glycemic Index Foundation of South Africa Endorsement logo (right). Source: Glycemic Index 2012

### *Associated Controversy*

There is a substantial amount of literature on GI, and for most topics associated with the measurement there are studies representing each opposing side. GI has been under fire in recent years for its validity as a scientifically sound tool. The limitation most concerning is the measurement's high amount of variability. There are several factors that can impact resulting values as seen in Table 1.2. The standard deviations of GI values are often so high that the food in question will overlap between categories. This can be seen in Wolever and others (2003) where spaghetti was given a GI value of  $47.6 \pm 18.1$ , and rice was given a GI value of  $68.7 \pm 22.6$ . Whelan and others (2010) found strong individual variation in responses. It was concluded that without knowledge of personal characteristic blood responses, use of general GI tables may be misleading. Lack of standardized methodology has also been criticized. Results for the same food often times differ between laboratories. This can be seen in Wolever and others (2008) where twenty eight labs tested the same two products, yet yielded very different results overall. Another difficulty faced is not having an official, standardized set of requirements for testing which results in a lack of precision across the board. The GI

concept is also accused of misrepresenting many foods. In applying the GI, the consumer is told to focus on low glycemic foods. This can be problematic when some healthy foods have a high GI, and some unhealthy foods have a low GI. This can be witnessed in Jenkins and others (1981) study where carrots were reported to have a GI of 92 (high), whereas sponge cake a GI of 46 (low). Additionally, the GI does not take into account the amount of carbohydrate typically consumed. Because GI testing uses 50g available carbohydrate, the GI for carrots appears high. This amount is much greater than one would eat in a normal sitting, and therefore a typical serving of carrots would not elicit a high glycemic response. Sausage on the other hand has a low GI due to the high fat content slowing gastric emptying. Although sausage has a low GI, a diet based around sausage and the like would not be recommended. There is also concern in that the concept only recognizes response to individual foods when in fact foods are rarely eaten alone, but in a mixed meal. There is a formula for calculating the GI of a mixed meal, but the validity of this is under question as well. Dodd and others (2011) tested seven foods individually, and then tested these same foods together in three separate mixed meals. A calculation using the individually tested food values was done to obtain a GI for each of the mixed meals, published GI values were used to calculate a GI for each of the mixed meals and GI values were tested for each mixed meal. Results showed that the calculation for a mixed meal, using obtained values and published values overestimated the GI of the mixed meals each time (Dodd and others 2011). As a result of these controversies, the general consensus for selection of carbohydrate based foods is to consider all properties of the food such as fat type and amount, fiber content, energy density, and total amount of carbohydrate (Aziz 2009).

Although no organizations in the United States currently support the use of GI, many health organizations around the world do. Diabetes Australia, Dietary Guidelines for Australians, Juvenile Diabetes Research Foundation of Australia, Canadian Diabetes Association, European Association for the Study of Diabetes and South African Diabetes Association all promote the use of GI for a healthy lifestyle (Glycemic Index Symbol 2012).

<b>Methodology Variation</b>	<b>Subject Variation</b>	<b>Test Food Variation</b>
<ul style="list-style-type: none"> <li>-Portion size of test food</li> <li>-Choice in reference foods</li> <li>-Method of blood sampling</li> <li>-Method of calculation</li> <li>-Time of day of testing</li> <li>-Type of glucose meter used</li> <li>-Differing replication methods</li> </ul>	<ul style="list-style-type: none"> <li>-Physical activity levels</li> <li>-Food consumption prior to fasting</li> <li>-Ethnicity</li> <li>-Hormonal status</li> <li>-Diseased versus healthy</li> <li>-Age</li> <li>-Gender</li> <li>-Body composition</li> <li>-Alcohol consumption</li> </ul>	<ul style="list-style-type: none"> <li>-Chemical structure of carbohydrate</li> <li>-Carbohydrate composition</li> <li>-Processing techniques</li> <li>-Method and degree of cooking</li> <li>-Starch nutrient interactions</li> <li>-Macronutrient/ Micronutrition composition</li> <li>-Degree of ripeness</li> <li>-Variety of food</li> </ul>

**Table 1.2 Sources of variation within glycemic index values for the same food. Sources: Aziz 2009, Hallfrisch and Behall 2000, Lin and others 2010, Mann and others 2010, Wolever and others 1991**

### *Similar Measurements*

In efforts of reducing variability in the GI measurement, similar concepts have emerged. One such measurement is glycemic load (GL). Glycemic load is the product of the GI and the amount of available carbohydrate. The result combines quality and quantity factors of carbohydrate in a food, whereas the GI does not account for quantity. There is a scale to classify glycemic load similar to that of GI as seen below in Table 1.3.



Category	Glycemic Load
Low	≤10
Medium	11-19
High	≥20

**Table 1.3 Glycemic Load Classification**

The glycemic load does present a limitation in that when converting from GI to glycemic load, the foods category may change depending on the serving size. This is positive when addressing healthy foods like watermelon with a GI of 72 (high), but when adjusted for serving size has a GL of 4 (low) [GL= .72 GI X 6g available CHO]. This can also be misleading in a food such as mashed potatoes with a GI of 74 (high) and a GL of 15 (med) [GL= .74 GI X 20g available CHO] (Aziz 2009).

Another concept developed was the glycemic glucose equivalent (GGE). The glycemic glucose equivalent gives the theoretical weight of glucose that would induce the glycemic response equivalent to that induced by a given amount of food. This more refined measurement takes into account the GI, food quantity and food composition (Miller-Jones 2007). Another constructive aspect of the glycemic glucose equivalent is that it is presented as g/serving which is familiar to consumers since nutrition labels are presented in this fashion (Aziz 2009). A limitation of the glycemic glucose equivalent is the fact that a large amount of time consuming testing would be required to generate proper tables which would be expensive. Also, this measure doesn't account for high

levels of other nutrients. A high fat product may result in a positive glycemic glucose equivalent, yet may not be an overall healthy choice (Aziz 2009).

### *Alternative Testing Methods*

Although there is standard methodology for *in vivo* GI testing, there are countless studies touting the validity of *in vitro* methods and animal testing to estimate the GI. Goni and others (1997) claimed to have an *in vitro* procedure to measure the rate of starch digestion that could then be used to estimate glycemic response to food. Englyst and others (1998) also produced an *in vitro* measurement said to reflect the glycemic response. Kraft Foods Inc. has recently developed a patent pending *in vitro* method for screening GI of foods (Nutraceuticals World 2010). There are many more studies that have used *in vitro* testing as well. Many studies manipulate factors that may have an effect on glycemic response, while others are estimating the GI of various foods. Yoon and others (1983) investigated the effect of phytic acid on the *in vitro* rate of starch digestibility and blood glucose response. Garcia-zaragoza and others (2010) used an *in vitro* method of GI testing to predict if there were changes in GI when pound cake is baked in a two cycle microwave toaster versus a conventional oven. O'Dea and others (1981) and Brand and others (1985) compared *in vivo* to *in vitro* results of GI testing, and found similar results between methods. *In vitro* methods are employed because they achieve rapid, cost effective results as opposed to *in vivo* methods.

Because the human body is such a complex operating system, exact replication *in vitro* is difficult. For example, high osmolality, high acidity and soluble fiber all slow gastric emptying in the body, and therefore reduce glycemic impact. These factors do

not have the same effect on the rate of digestion *in vitro*. *In vitro* testing for GI has yet to be proven a valid test (Foster-Powell and others 2002).

### *Associated Conditions*

Low GI diets have been linked to prevention and treatment of many common health conditions in today's society. GI has been associated with diabetes, cardiovascular disease, obesity, cancer and athlete performance. These controversial topics have resulted in an abundant amount of studies in an attempt of proving significance in either direction. Clinical application currently remains the subject of debate (AICR 2009).

### *Diabetes*

Many propose that a diet based on low GI foods will assist in maintaining blood glucose control in diabetes (AICR 2009). It is also hypothesized that hyperglycemia and hyperinsulinemia which results after eating high GI foods leads to beta cell dysfunction over time, and therefore a low GI diet would support diabetes prevention (Ludwig 2002). Although some studies show improvements in blood glucose levels from low GI diets, these studies are not without limitations. Limitations include short trial periods, too few subjects and overall inconsistent results. Some of these studies are epidemiological studies, or are based on surveys. There are no long term studies depicting the effect of low GI diets on blood sugar levels. Furthermore, there is no evidence that a low GI diet is superior to other nutritional interventions (AICR 2009). The American Diabetes Association recognized that low GI foods in fact reduce postprandial glycemia, but concluded that "evidence for long term benefits from low GI diets haven't been established, and data reveals no clear trend in outcome benefits (ADA 2003)." The

Canadian Diabetes Association and Glycemic Index foundation of Australia however, considers the GI as a useful concept in managing diabetes (CDA 2012, The Glycemic Index Symbol 2012).

#### *Cardiovascular Disease*

Low GI diets have been associated with lowering blood triglycerides and improving insulin sensitivity, whereas high glycemic load diets have been linked to insulin resistance syndrome, high blood pressure, lowering of HDL cholesterol, pro-inflammatory activity and abnormal blood lipids. Studies showing these associations between a low GI diet and cardiovascular disease risk factors however lack long term research, and exhibit inconsistent findings (AICR 2009). There is however strong evidence that consumption of whole grains, legumes, vegetables and fruits reduce the risk of cardiovascular disease (Mann and others 2007).

#### *Obesity*

There are many weight loss systems on the market based on the GI concept. Many claim that low GI foods result in higher satiety and vice versa. Many studies have investigated this theory, however inconsistent findings have resulted. There have been no long term investigations. The Academy for Nutrition and Dietetics had the following response to the book "The G.I. Diet Clinic": "Bottom line The Academy of Nutrition and Dietetics doesn't subscribe to the Glycemic index theory for weight loss" (AND 2012).

#### *Cancer*

There are fewer studies between cancer and GI to date, but published work that exists has inconsistent conclusions. Cancers that have been studied with a possible connection to GI are breast, colon, pancreatic, ovarian, and endometrial (AICR 2009).

### *Athlete Endurance*

It is known that consuming carbohydrate before, during, and after strenuous exercise improves athlete performance. Some claim that consuming low GI carbohydrates before exercise will optimize performance by providing slow and sustained energy release throughout a workout. Several studies have been conducted comparing performance, or work output after a pre-workout meal from high and low GI carbohydrate. Overall, these studies results are inconsistent, and it is recommended to let individual experience dictate consumption (Donaldson and others 2010).

### *Positions on Glycemic Index*

The following are positions on the GI concept from various organizations throughout the world.

- The American Diabetes Association's position on glycemic index is that total amount of carbohydrate is more important than the source or type (ADA 2003).
- The Food and Agriculture Organization and World Health organization collaboratively concluded that the glycemic index can most appropriately be used when considering between similar carbohydrate foods (Mann and others 2007).
- The Academy for Nutrition and Dietetics reported that if the use of glycemic index is proposed as a method of meal planning, the RD should advise on the conflicting evidence of effectiveness of this strategy. Studies

comparing high versus low GI diets report mixed effects on HbA1C (AND 2012).

- The American Institute for Cancer Research believes that it is premature to incorporate the GI concept into dietary recommendations for the public at this time (AICR 2009).
- The Glycemic Index Foundation of Australia claims that low GI diets:
  - Help to fill you up and keep you feeling satisfied for longer, avoiding over eating or too much snacking.
  - Lower your insulin levels which makes fat easier to burn and less likely to be stored.
  - Help you to lose body fat and maintain lean muscle tissue.
  - Reduce your triglycerides, total and 'bad' (LDL) cholesterol.
  - Increase your levels of 'good' (HDL) cholesterol.
  - Reduce your risk of developing type 2 diabetes.
  - Help to manage your blood glucose levels and reduce your risk of developing diabetes complications.
  - Reduce your risk of developing cardiovascular disease.
  - Reduce your risk of developing some cancers
  - Reduce your risk of developing certain eye diseases.
  - Improve your skin
  - Sustain your energy levels longer, improving both mental and physical performance. (The Glycemic Index Symbol 2012)



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## **Chapter 2 - A Comparison of the Glycemic Index of Sorghum and Other Commonly Consumed Grains**

## **Introduction**

Sorghum is a plentiful crop in the United States with approximately 300-500 million bushels at disposal each year (USDA 2011). Because sorghum is a gluten free grain, its utilization for human consumption in the United States remains on the rise as the gluten free market continues to expand. The gluten free market is expected to reach \$6 billion in sales by 2015, doubling last year's mark (Food Product Design 2011). In order to further utilize sorghum's potential in commercialized food products, there is a need for nutrition based research. There is limited published research on its digestion and metabolism. In order to gain a better understanding of how sorghum is metabolized in the body, this study had the following objectives.

- Establish a glycemic index category for sorghum in a baked product.
- Compare sorghum's glycemic index to other commonly consumed grains in the United States.
- Investigate if an optimal particle size exists in relation to a lower glycemic response.
- Investigate if a correlation between percent starch damage and glycemic index exists.

Furthermore, meeting these objectives will provide product developers the useful information to assist in producing value-added sorghum based foods. This in turn will benefit the consumer in efforts to meet their needs.



## **Materials and Methods**

### **Grain Milling**

Four grains were obtained for grinding, and commercial all-purpose wheat flour was purchased (HyVee Inc., West Des Moines, IA). White sorghum of Fontanelle variety D-1000-7 (USDA-Center for Grain and Animal Health Research, Manhattan, KS), Karl 92 hard red winter wheat (USDA-Center for Grain and Animal Health Research, Manhattan, KS), Dynagro 57V15 corn (USDA-Center for Grain and Animal Health Research, Manhattan, KS), and brown rice (HyVee Inc., West Des Moines, IA) were ground into flour using a UDY Cyclone Lab Sample Mill (UDY Corp., Fort Collins, CO). The UDY mill produces uniform grinding with complete recovery of samples by employing high speed rotation of an impeller, and air current that forcefully throws particles against and around a grinding ring. Once particles become small enough, subsequent flour exits through a screen via air current. Screen size thereby determines particle size of flour. The wheat, corn, and brown rice samples were milled with a .5 mm screen. Sorghum was milled into three samples using a .5 mm screen (fine), a 1 mm screen (intermediate), and a 2 mm screen (coarse). All seven flour samples were placed in re-sealable bags (Ziploc Brand, New Brunswick, NJ) and stored in a commercial freezer held at approximately 26°F until used.

### **Physical Analysis of Flour**

#### *Particle Size*

A Beckman Coulter LS™ 13 320 SW Dry Powder System Laser Diffraction Particle Size Analyzer (Beckman-Coulter, Inc., Miami, FL) was used to determine the particle size distribution of the milled flours. The flour was placed into the load cell until it

was approximately 2/3 full. The cell was then loaded into the Tornado™ Dry Powder Dispersing attachment for the instrument, and measurements were taken. The LS™ 13 320 SW uses light scattering properties to determine the particle size distribution.

### ***Starch Damage***

Starch damage was determined using the Megazyme Starch Damage Assay Procedure, K-SDAM 02/2008, AACC Method 76-31 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). In this procedure, damaged starch granules are hydrolyzed to maltosaccharides and alpha-limit dextrans through a highly controlled treatment with purified fungal alpha-amylase. This leads to nearly complete solubilization of the damaged starch granules, while minimizing breakdown of undamaged granules. The reaction was terminated with dilute sulphuric acid. Aliquots were subsequently treated with excess levels of purified amyloglucosidase to give complete degradation of starch dextrans to glucose. The resulting solution is reacted with glucose oxidase/peroxidase reagent, and the glucose concentration was determined colorimetrically. Reported values are presented as a percentage of flour weight on an “as is” basis.

### ***Preliminary Work***

A test bake was completed for all muffin types to ensure that the formulas would yield muffins with physical integrity and that were edible. Because flour from different grains and particle sizes hydrate differently, water levels for each formulation was determined subjectively on account of achieving a batter like consistency. Oh and others (1985) found that as particle size decreased, more water was required in noodle production. Pomeranz and others (1977) observed that water absorption increased with extraction rate, probably due to an increase in protein and fiber content. Muffins were

baked and frozen according to the procedure outlined in “Muffin Formulation and Preparation” below. The initial flours tested were three particle sizes of Fontanelle variety D-1000-7 sorghum, corn, brown rice, whole wheat, all-purpose flour, and a sumac variety of sorghum. Because sumac has a pigmented pericarp and tannins it exhibits a dark brown color and strong flavor in food. Using a white, mild flavored sorghum such as the Fontanelle variety in commercial products is more likely as it wouldn't lend these characteristics. For this reason, sumac was eliminated as a treatment. Proximate analysis was run on the test muffins to calculate grams of available carbohydrate. After calculating and weighing out the standard 50g available carbohydrate serving, this amount was determined to be an unreasonably large serving for one sitting. A 20g available carbohydrate serving was then chosen for testing purposes.

### ***Muffin Formulation and Preparation***

All seven muffin formulations were composed of 300g flour, 4.5g baking powder (Clabber Girl, Terre Haute, IN), 3.5g salt (Morton, Chicago, IL), and tap water. Water levels varied on account of flour type and particle size. A test bake was done to find optimal water levels. Amounts of water used per formula are listed in the table below (Table 2.1). Dry ingredients (flour, baking powder, and salt) were measured and mixed together by hand for 30 seconds. Water was added to the dry mixture and blended by hand for 1 minute. Sixty grams of batter was scaled out into each hole of a twelve hole muffin tin. Muffins were baked in a pre-heated oven at 350°F. Muffins made from all-purpose flour, whole wheat flour, corn flour and rice flour were baked 30-35 minutes, whereas those made from sorghum flour baked 15 minutes. Muffins were cooled in the

pan for about 10 minutes, and then removed onto a cooling rack for an additional 2 hours, or until muffins reached room temperature. Muffins were placed in re-sealable bags (Ziploc Brand, New Brunswick, NJ), labeled and stored in a commercial freezer held at approximately 26°F until human trials. Sample corn flour and all-purpose flour muffin treatments can be seen in Figure 2.1.

<b>Flour Type</b>	<b>Water Amount (g)</b>
Sorghum (Fine)	415 g
Sorghum (Intermediate)	350 g
Sorghum (Coarse)	315 g
Corn	400 g
Rice	385 g
Whole wheat	385 g
All-purpose	375 g

**Table 2.1 Muffin Formula Water Levels**



**Figure 2.1 Muffin treatments made from all-purpose flour (bottom) and corn flour (top)**

## **Proximate Analysis**

### ***Percent Moisture***

The moisture contents of the flours were measured using the Association of Official Analytical Chemists (AOAC) approved method 930.15 on an “as is” basis. The procedure determines the dry matter of the sample by oven drying at 135°C for 2 hrs. Moisture is evaporated from the sample during the drying, and then dry matter is determined gravimetrically as the residue remaining after drying. The moisture is then calculated by subtraction of dry matter from the whole sample.

Calculation:

$$\text{Moisture Content} = \frac{(\text{Wet Sample Weight} - \text{Dry Sample Weight})}{\text{Wet Sample Weight}} \times 100$$

### ***Percent Crude Protein***

The protein contents of the flours were measured using AOAC approved method 990.03: Nitrogen Determination by Combustion on an “as is” basis. Nitrogen in the sample is freed by combustion at high temperatures in pure oxygen, and then measured by thermal conductivity. This value is converted to the equivalent protein by the default numerical factor of 6.25.

### ***Percent Crude Fat***

The fat contents of the flours were measured using AOAC approved method 920.39 on an “as is” basis. This method determines crude fat in the samples by ether extraction with subsequent solvent evaporation. The fat content is reported as a percentage of the original sample weight.

### ***Percent Crude Fiber***

The crude fiber content of the flours was measured using the Ankom Method, based on AOAC 962.09 on an “as is” basis. The Ankom crude fiber solvent solubilizes non-fiber components of the flour, and then the sample is filtered, rinsed and dried to determine the crude fiber content. Crude fiber is reported as a percentage of the original sample weight.

### ***Percent Ash***

The ash contents of the flours were measured using AOAC approved method 942.05 on an “as is” basis. About two grams of each sample was weighed into a porcelain crucible and placed in a temperature controlled furnace preheated to 600°C. The sample was held at this temperature for 2 hours. The crucible was then transferred directly to a desiccator, cooled and then weighed. Ash content is reported as a percentage of the whole sample.

### **Available Carbohydrate**

Total carbohydrate for each muffin type was calculated by difference using proximate analysis results from 100g samples.

Calculation:

$$\text{Total Carbohydrate} = 100 - (\text{Protein} + \text{Fat} + \text{Water} + \text{Ash} + \text{Fiber})$$

Twenty grams available carbohydrate for each muffin type was then calculated by solving for X.

Calculation:

$$\text{Muffin Treatment Weight (g)} = \frac{\text{Total Carbohydrate (g)}}{20 \text{ g Available Carbohydrate}} \times \frac{100 \text{ g sample}}{X}$$

Final serving size in grams for each muffin type are given in Table 2.2.

<b>Flour Type</b>	<b>Serving Size (g)</b>
Sorghum (Fine)	53.3
Sorghum (Intermediate)	47.8
Sorghum (Coarse)	55
Corn	49.1
Rice	44.3
Whole wheat	55
All-purpose	45

**Table 2.2 Serving Size of Muffins for 50g Available Carbohydrate**

### **Subjects**

Eight healthy volunteers (ages 18-40) participated in the study. Volunteers were recruited among Kansas State University students. All subjects gave their signed consent, and were aware of all parameters surrounding the study. Approval of the study was obtained from the Institutional Review Board (IRB# 3941.2). Subjects were asked to maintain their regular lifestyle throughout the entirety of the study, avoiding any extreme behaviors. Each subject selected two weekly time slots for their testing.

### **Evaluation of Glycemic Index**

The seven treatments included muffins made from all-purpose flour, whole wheat flour, brown rice flour, corn flour and three sorghum flours. All treatments were labeled with codes 101-107. Subjects received two treatments per week by random order with at least a forty eight hour period in between. Each treatment was given twice as well as one dosage of a 20g dextrose drink (reference food) over an eight week period.

Subjects were required to fast ten hours prior to each visit, but were permitted to consume unlimited drinking water throughout testing. A weighed portion of muffin containing 20g of available carbohydrate that had been thawed to room temperature overnight was administered at each testing session. Two finger prick samples were taken for capillary blood analysis by a lab assistant at 0 (fasting), 30, 45, 60, 90 and 120 minutes after consumption and averaged. An YSI 2300 Glucose Oxidase Analyzer (Yellow Spring's Instruments, Yellow Springs, OH) was used to measure blood glucose concentrations.

### **Calculation of Glycemic Index**

The incremental area under the glycemic response curve (iAUC) was constructed using the trapezoid model with fasting levels as the baseline by GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA). Area below the baseline was excluded. The GI values were calculated by dividing the iAUC for the test food (muffin) by that for the standard (20g dextrose drink) and multiplied by 100. The GI for each treatment was calculated as the mean from the respective average GIs of the eight volunteers.

Calculation:

$$\text{Glycemic Index} = \frac{\text{iAUC (treatment)}}{\text{iAUC (standard)}} \times 100$$

### **Statistical Analysis**

Two replications of each treatment were used in a randomized block design. GI values were analyzed using SAS, Software Release 9.3 (SAS, Institute Inc., Cary, NC,



2011). Difference in response between grains, flour particle size and proximate analysis composition of muffins was analyzed for significance. When treatment effects were found significantly different, the least square means with Dunnet's P-values were used to differentiate treatment means. A level of significance was reported at  $p < 0.05$ .

Regression analysis was done to find whether a correlation between starch damage and glycemic response exists using Excel (Microsoft Office Excel, Microsoft Corporation, Redmond, WA).

## **Results and Discussion**

### ***Physical Analysis of Flour***

#### ***Particle Size***

Particle size of flour represents the degree of volume of particles and the total exposed surface area of particles dispersed throughout flour (Pratt 1978). Resulting particle size distribution of flour is a result of kernel hardness, moisture content, kernel mass and milling methods (Campbell and Muhamad 2007). A variation in particle size distribution per grain is present in the current study. The finely milled sorghum, corn, brown rice and whole wheat were all ground through a .5 mm screen, yet exhibited different particle size distributions. Values for both mean d90 particle size distribution and mean particle size distribution are listed in Table 2.3. Particle size distribution reported as d90 values indicate that 90% of the volume of flour particles is less than the given values in microns. Mean particle size distribution represents the overall average particle size of the flour. A significantly lower glycemic response was found from the muffin treatments made of intermediately milled sorghum flour compared to other

sorghum flours tested (Table 2.5). Sorghum muffins made from the intermediate particle size flour resulted in the lowest glycemic response of all grains tested. This leads to a hypothesis that optimum particle size is grain specific in terms of metabolism.

Many researchers have proposed that as particle size decreases, more surface area is exposed to digestive enzymes, and therefore a higher glycemic response will result. Holt and Miller (1994) showed a higher glycemic response to wheat as particle size decreased by testing equal carbohydrate portions of baked whole grains and cracked grains, and muffins from coarse and fine wholemeal flours. Because cooking methods and form of food also affect glycemic response, this association does not prove a cause and effect relationship. Two studies by O'Dea and others (1980), and Collier and O'Dea (1982) compared glucose responses to whole brown rice and ground brown rice, and concluded that the actual form of complex carbohydrate is critical in determining the metabolic response. The ground brown rice elicited significantly higher glucose responses in both cases. O'Donnell and others (1989) studied the effects of particle size on glycemic response within a mixed meal. A test meal of scones made from either coarse or finely milled wheat flour, cheddar cheese, butter, tea, and milk was served by standard testing protocol. It was observed that the meal containing the scone made from coarse flour resulted in lower plasma glucose and insulin concentrations than that containing the scone made from fine flour (O'Donnell and others 1989). Heaton and others (1988) found significantly higher glucose response from scones made of finely milled wheat flour versus coarser milled wheat flour. Cornmeal and oat flours did not reveal a significant difference, but still reflected an increased response as particle size decreased. Corn products are known to have a wide range of glucose and

insulin responses depending on cultivar, form, processing and amylose, amylopectin ratio (Hallfrisch and Behall 2000).

There are however, inconsistencies with this hypothesis. Behall and others (1999) found no significant differences when comparing particle size and glycemic response from breads made from refined wheat flour, coarse whole grain flour, and fine whole grain flour. Also, according to this hypothesis, the coarser milled sorghum flour would have resulted in the lowest glycemic response, yet it had a higher response than the intermediately milled flour. Furthermore, brown rice had the smallest particle size of all flours, yet revealed a low glycemic response of  $37 \pm 18$ . Rice is also a grain known to have a wide range in glycemic response due to starch content (Hallfrisch and Behall 2000). Inconsistencies in findings and high standard deviations may be attributed to the fact that metabolism is highly variable and responses may be altered on account of cultivar, form, amount and method of cooking, as well as the health characteristics, age and gender of subjects studied (Hallfrisch and Behall 2000). High standard deviation in particle size of corn flour is due to the lipid content in the flour which caused caking in the Beckman Coulter LS™ 13 320 SW Dry Powder System Laser Diffraction Particle Size Analyzer equipment during analysis.

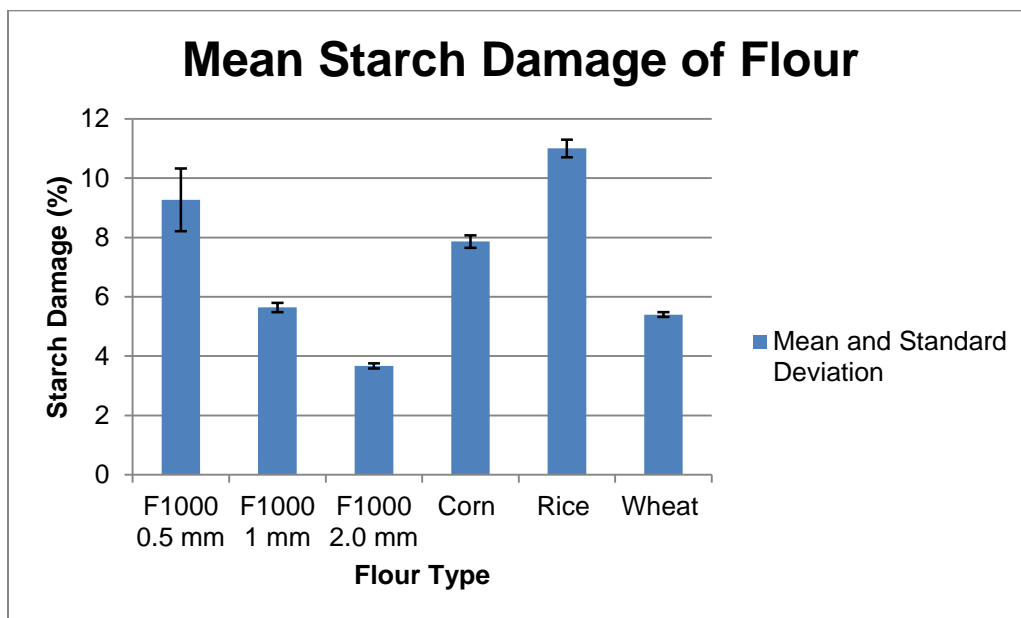
<b><i>Flour Type</i></b>	<b><i>Mean d90 Flour Particle Size Distribution (μm)</i></b>	<b><i>Mean Particle Size Distribution (μm)</i></b>
<b>Sorghum (fine)</b>	190.85 ± 0.55	82.15 ± 1.10
<b>Sorghum (intermediate)</b>	394.37 ± 11.38	166.90 ± 3.96
<b>Sorghum (coarse)</b>	731.69 ± 42.64	303.27 ± 15.45
<b>Corn</b>	246.62 ± 47.07	92.64 ± 16.14
<b>Rice</b>	154.22 ± 2.59	67.90 ± 0.41
<b>Wheat</b>	307.43 ± 2.17	325.11 ± 304.49

**Table 2.3 Mean d90 particle size distribution and mean particle size distribution of finely milled sorghum flour, intermediate milled sorghum flour, coarsely milled sorghum flour, corn flour, brown rice flour, and whole wheat flour**

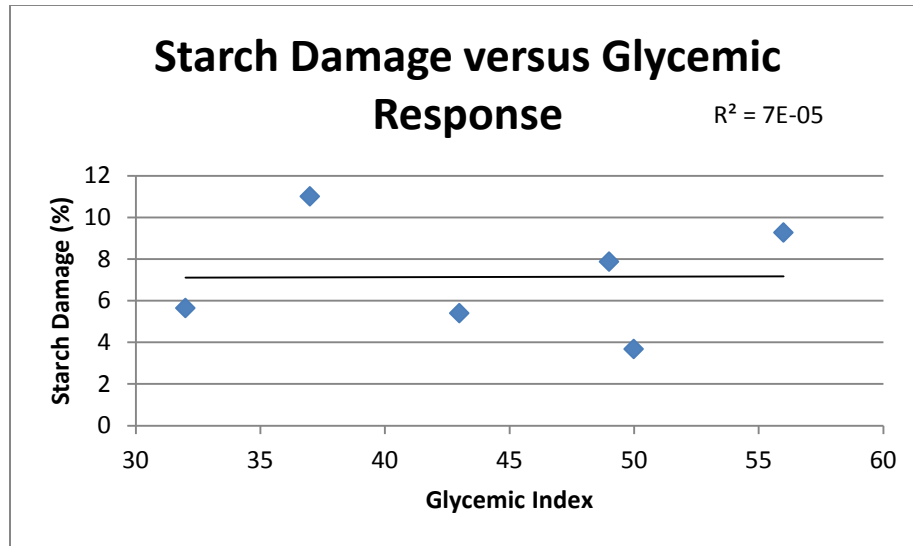
### ***Starch Damage***

When grains are ground or milled into flour, intact starch granules become damaged, leaving starch granules that have been fractured, shattered, or chopped (Chen and D'Appolonia 1986). Grain hardness is a major contributor to starch damage that results from milling. In soft wheat, starch granules are loosely bound within the kernel and are easily released whereas starch granules in hard wheat are tightly bound within the protein matrix, and are much more susceptible to damage as the endosperm is fractured (Evers and Stevens 1985). One would also assume that the less abrasion applied to a grain during milling, the less final starch damage would result. In the current study as particle size of the sorghum flours increased, starch damage decreased (Figure 2.2). Brown rice flour revealed the highest percent starch damage among all flours (Figure 2.2).

The greater the starch damage in flour, the more susceptible the damaged granules are to enzymatic attack (Pylar 2009). Starch damage is also associated with an increase in water absorption by starch granules (Khan and Shewry 2009). Alpha-amylase both from salivary glands and the pancreas cleave  $\alpha$ -1, 4 glucose bonds in amylose during digestion. Amylose becomes more readily digestible in a food as it has been processed. Heat and hydration rupture starch granules and facilitate enzyme hydrolysis (Berdanier, 1976). These factors suggest a possible connection between starch damage and glycemic response. No significant correlation however, between the amount of starch damage and glycemic response was present as seen in Figure 2.3.



**Figure 2.2 Percent starch damage of finely milled sorghum flour, intermediate milled sorghum flour, coarsely milled sorghum flour, corn flour, brown rice flour, and whole wheat flour**



**Figure 2.3 Correlation between percent starch damage of finely milled sorghum flour, intermediate milled sorghum flour, coarsely milled sorghum flour, corn flour, brown rice flour, and whole wheat flour and glycemic response to muffin treatments made from these flours**

### *Proximate Analysis*

Although values for total carbohydrate in Table 2.3 differ, all muffin treatments were administered as 20g available carbohydrate. Whole wheat muffins contained the highest level of protein, and corn muffins had the highest percent fat content (Table 2.4). Muffins made from the coarsely milled sorghum flour had significantly higher fiber than all other muffins with the exception of the whole wheat muffins (Table 2.4). This is an inconsistent and unexpected relationship as all sorghum flours in this study were milled from the same lot of sorghum. The different particle sizes of the sorghum flours could have caused a difference in reaction rates and results between proximate analysis tests. Because serving size of test food was calculated from these results, there could be a degree of error present.

Composition of a food system such as the amount of fiber, fat, protein, calcium, acids and enzymes can alter gastric emptying and glycemic response due to various interactions (Vosloo 2005). For this reason it was important to formulate the muffins with minimal ingredients. All muffin treatments contained only flour, water, baking powder and salt. No oil was applied to muffin tins before baking.

Jenkins and others (1981) found a significantly negative relationship for both fat and GI, and protein and GI. This does not apply to the current study as the whole wheat muffins and corn muffins have the highest levels of naturally occurring protein and fat, yet did not exhibit lower glycemic responses than the other muffins tested.

Jenkins and others (1978) added various types of fiber to test foods and witnessed a significantly reduced blood glucose concentration during the glucose tolerance tests, and reduced serum insulin concentrations. Wolever (1990) studied naturally present fiber content in foods and their subsequent glycemic response, and revealed that total dietary fiber was significantly related to the GI of foods. Lakshmi and Vimala (1996) tested the glycemic response of three sorghum recipes comparing dehulled versus whole grain sorghum for each recipe. The whole grain sorghum recipes resulted in a significantly lower glycemic response than the recipes made from dehulled sorghum. Again, this did not occur with the coarse sorghum flour muffins in the current study despite them having the highest fiber content. All protein, fat, and fiber present in the muffins are merely native to the flour used, and may not be at high enough levels to alter

a response. Also, other factors may have interfered such as type and degree of starch present.

<b>Muffin Type</b>	<b>%Moisture</b>	<b>% Crude Protein</b>	<b>% Crude Fat</b>	<b>% Crude Fiber</b>	<b>% Ash</b>	<b>% Total Carbohydrate</b>
<b>Sorghum (fine)</b>	55.62 ± 0.20 <sup>a</sup>	4.38 ± 0.20 <sup>e</sup>	0.79 ± 0.01 <sup>c</sup>	0.38 ± 0.01 <sup>c</sup>	1.33 ± 0.01 <sup>cd</sup>	37.50 ± 0.20 <sup>e</sup>
<b>Sorghum (intermediate)</b>	50.25 ± 0.04 <sup>c</sup>	4.87 ± 0.05 <sup>d</sup>	0.97 ± 0.02 <sup>b</sup>	0.52 ± 0.03 <sup>b</sup>	1.54 ± 0.01 <sup>ab</sup>	41.80 ± 0.14 <sup>c</sup>
<b>Sorghum (coarse)</b>	55.47 ± 0.01 <sup>a</sup>	5.05 ± 0.02 <sup>c</sup>	1.02 ± 0.01 <sup>b</sup>	0.70 ± 0.02 <sup>a</sup>	1.34 ± 0.02 <sup>cd</sup>	36.41 ± 0.00 <sup>f</sup>
<b>Corn</b>	52.73 ± 0.21 <sup>b</sup>	3.74 ± 0.01 <sup>g</sup>	1.17 ± 0.00 <sup>a</sup>	0.28 ± 0.02 <sup>d</sup>	1.38 ± 0.02 <sup>c</sup>	40.72 ± 0.18 <sup>d</sup>
<b>Rice</b>	48.81 ± 0.07 <sup>d</sup>	4.15 ± 0.03 <sup>f</sup>	0.29 ± 0.01 <sup>d</sup>	0.06 ± 0.0 <sup>e</sup>	1.50 ± 0.02 <sup>b</sup>	45.20 ± 0.07 <sup>a</sup>
<b>Wheat</b>	52.96 ± 0.05 <sup>b</sup>	8.05 ± 0.05 <sup>a</sup>	0.31 ± 0.00 <sup>d</sup>	0.65 ± 0.02 <sup>a</sup>	1.58 ± 0.01 <sup>a</sup>	36.40 ± 0.03 <sup>f</sup>
<b>All-purpose</b>	47.71 ± 0.10 <sup>d</sup>	6.80 ± 0.02 <sup>b</sup>	0.14 ± 0.00 <sup>e</sup>	0.02 ± 0.00 <sup>e</sup>	1.31 ± 0.01 <sup>d</sup>	44.0 ± 0.07 <sup>b</sup>

\*Means with the same letter are not significantly different ( $P \leq 0.05$ ). Characteristics not sharing the same letter were significantly different ( $p < 0.05$ ).

**Table 2.4 Percent composition of 100 g muffin treatments made from finely milled sorghum flour, intermediate milled sorghum flour, coarsely milled sorghum flour, corn flour, brown rice flour, whole wheat flour, and all-purpose flour**

### *Glycemic Index*

Results for GI from smallest to largest is sorghum (intermediate), brown rice, whole wheat, all-purpose flour, corn, sorghum (coarse), and sorghum (fine) as seen in Table 2.5. Muffins made from intermediately milled sorghum flour and brown rice flour resulted in a significantly lower blood glucose response ( $p < 0.05$ )



than all other muffins tested. Each GI value is reported according to blood glucose response to 20g available carbohydrate. A 20g sample was used as opposed to the standard 50g sample because this amount is more realistic of what one may consume in a typical sitting. An example iAUC for the fine sorghum muffin is seen in Figure 2.4.

Glycemic response to rice is known to vary widely on account of starch structure content. Typically the higher the ratio of amylose to amylopectin in grains, the lower the resulting glycemic response. This is due to the highly branched structure of amylopectin being susceptible to enzymatic attack as opposed to the linear structure of amylose. Behall and others (1988) found subjects to have significantly lower glucose and insulin responses after crackers composed of amylose compared to those made of amylopectin. Behall and others (1989) conducted a similar study comparing the responses to amylose after a five week period versus amylopectin consumption after a five week period, and found similar results. Panlasigui and others (1991) however, concluded that amylose content alone was not a good predictor of starch digestion rate or glycemic response. Sorghum's amylose content is typically only 20-30% which would indicate a higher glycemic response based on this mechanism alone (Dicko and others 2006).

There is much debate on the digestibility of sorghum in terms of glycemic response. Powell and others (2002) reported roasted jowar bread, or sorghum bread as having a GI of  $77 \pm 8$  in the 2002 edition of the International Table of Glycemic Index and Glycemic Load. Mani and others (1993) also found sorghum

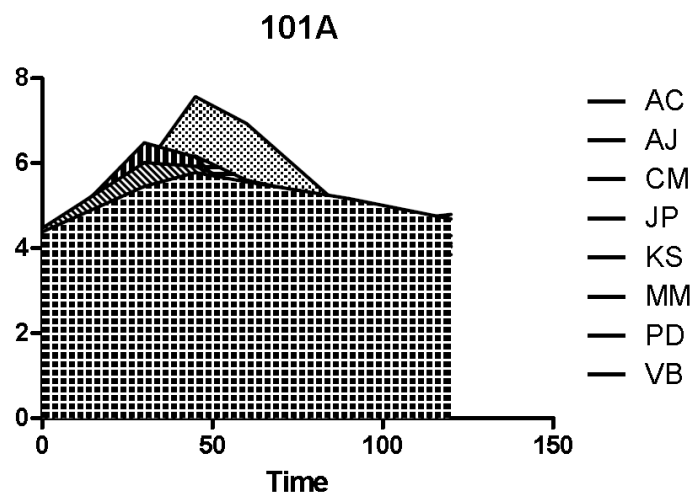
to have an identical GI after testing six commonly consumed sorghum foods of India. In both cases sorghum is classified as having a high GI. Abdelgadir and others (2004) compared the glycemic effect on individuals with diabetes of a sorghum based flatbread and porridge against wheat pancakes, millet porridge, and maize porridge. Results indicated the wheat pancakes and millet porridge had significantly lower glycemic effect than the maize and sorghum foods.

One mechanism suggesting that sorghum is slowly digested is its tannins. Dyke and Rooney (2007) reported that tannins bind to protein, carbohydrates, and minerals which decrease the digestibility of these nutrients. Thompson and others (1983) found a negative correlation between GI and concentration of polyphenols after testing thirteen foods. In the current study sorghum muffins with optimal particle size was found to have a lower glycemic response as opposed to the other grains tested, however the standard deviations of the results remain high so further research is needed. There is a plethora of *in vitro* studies on sorghum digestibility that suggest sorghum has slowly digested starch. From these studies there are several theories based on the fact that sorghum is slowly digested such as a protein-starch interaction, resistant starch, and acid-starch interactions. The mechanism of the starch-protein interaction can be explained by protein disulfide bond cross linking involving the kafirin prolamins in the protein matrix around the starch granules which reduces starch digestibility (Taylor and Emmambux 2010).

	<i>Sorghum (fine)</i>	<i>Sorghum (intermediate)</i>	<i>Sorghum (coarse)</i>	<i>Corn</i>	<i>Rice</i>	<i>Wheat</i>	<i>All- purpose</i>
GI	56 ± 32.7 <sup>b</sup>	32 ± 16.8 <sup>a</sup>	50 ± 26.0 <sup>ab</sup>	49 ± 29.1 <sup>ab</sup>	37 ± 18.0 <sup>a</sup>	43 ± 22.8 <sup>ab</sup>	44 ± 21.8 <sup>ab</sup>

\*Means with the same letter are not significantly different ( $P \leq 0.05$ ). Grains not sharing the same letter were significantly different ( $p \leq 0.05$ ).

**Table 2.5 Mean glycemic response values to muffin treatments made from finely milled sorghum flour, intermediate milled sorghum flour, coarsely milled sorghum flour, corn flour, brown rice flour, whole wheat flour, and all-purpose flour**



**Figure 2.4 iAUC of Fine Sorghum Muffin Treatment**

## Conclusions

Overall, this study demonstrated key metabolic properties of sorghum in terms of glycemic impact. Sorghum fell in the GI range of low to intermediate depending on the particle size of flour used. When compared to other grains tested, sorghum product from intermediately milled flour exhibited a significantly lower glycemic response with the exception of brown rice. Intermediately milled sorghum flour resulted in the lowest

glycemic response when comparing three particle sizes of sorghum flour. No correlation was found between percent starch damage in flour and GI of a baked product. These results can assist in the product development process to advance the quality of healthy, gluten free sorghum based foods for consumers.

### **Recommended Future Work**

Because little has been documented regarding digestive properties of sorghum, this study is in preliminary stages on the subject, and much future work is needed. Testing the GI of sorghum on a diabetic population would be beneficial because characteristics such as insulin sensitivity and glucose tolerance status influence the glycemic response to food (Brouns and others 2005). Low GI foods are of most use to this population, therefore their reaction to sorghum would be of interest especially for marketing purposes. Additionally, an exact range of intermediate particle size of sorghum flour needs to be defined. This will aid product developers in creating low GI sorghum products. Further investigations between tannin levels, resistant starch levels and protein-starch interactions of sorghum and GI are needed. It is suspected that higher tannin sorghums will result in an even lower GI. Research on sorghum's resistant starch and protein-starch interactions may uncover the mechanism for low glycemic impact of sorghum.

### **Study Limitations**

The formulations in this study were kept very basic on account of comparison between grains, but it is recognized that in a commercialized sorghum product other nutrients such as fat, sugar and the like would be added and subsequently affect the

glycemic response. Thus the GI found for sorghum in this study is not a generalized value for all sorghum products.

Regarding the study design, although the study initially had ten subjects, two subjects discontinued participation before the study's end. Also, only 20g available carbohydrate was administered during testing opposed to the standard 50g sample because this amount is more realistic of what one may consume in a typical sitting. Published standards however, recommend a minimum of 25g available carbohydrate to an ideal 50g available carbohydrate to be administered (Brouns and others 2005). Due to time constraints the glucose drink or reference food was not administered in duplicate or triplicate as recommended.

Further physical analysis of flour such as amylose to amylopectin ratio and total resistant starch would have contributed to a more complete understanding of the glycemic impact of sorghum. Finally, the milling methods of the purchased all-purpose flour are unknown. As such, direct comparisons for particle size and starch damage was not analyzed for this treatment.

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