# IN VITRO STARCH DIGESTIBILITY AND ESTIMATED GLYCEMIC INDEX OF SORGHUM PRODUCTS

A Thesis

by

ANGELINA DE CASTRO PALOMINO SILLER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Food Science and Technology

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Approved by:

| | |
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## ABSTRACT

In vitro Starch Digestibility and Estimated Glycemic Index of Sorghum Products.
(May 2006)
Angelina de Castro Palomino Siller, B.S., Instituto Tecnológico y de Estudios Superiores de Monterrey
Chair of Advisory Committee: Dr. Lloyd W. Rooney

Fractions from white and tannin sorghums were processed into extrudates, thick porridges, and breads. The effects of sorghum type and fraction on the in vitro starch availability of the products were evaluated, and the estimated glycemic indexes (EGI) of the products were obtained.

Sorghum extrudates were significantly more slowly digested than corn meal extrudates for all preparation methods (whole, cracked and decorticated kernels). Furthermore, tannin extrudates were less digestible than white sorghum extrudates. The soft endosperm nature of the tannin sorghum limited friction formation inside the extruder, reducing starch gelatinization. On the other hand, condensed tannins also interfered with the starch availability for digestion. White sorghum was more “suitable” for extrusion, giving extrudates with higher starch degradation and expansion than the tannin sorghums. However, tannin sorghums also gave acceptable products offering the benefit of lower EGI values.

Sorghum porridges were more slowly digested than a corn flour porridge when using whole and decorticated flours. In addition, tannin sorghum porridges had a lower starch digestibility compared to all the samples. Tannin sorghum flours produced soft porridges with enhanced initial starch digestibility. However, condensed tannins seemed to offset the starch digestion by limiting starch

availability. All sorghum porridges had significantly lower EGI values than the corn porridge.

Extrudates and porridges had reduced starch digestibilities and EGI values when using whole grains compared to using the decorticated fractions. This was observed in both the white and the tannin sorghum. Therefore, whole-grain products from sorghum have health benefits attributed to whole grain foods and slower digesting starches; for instance, prevention and treatment of diseases such as diabetes, insulin resistance, obesity, cardiovascular disease, and some types of cancer.

When 12% of tannin bran was added to a wheat bread formulation, a slower rate of starch digestion was observed compared to a wheat bread. The high concentration of non-starch components of the bran (i.e. dietary fiber, condensed tannins) affected starch digestion. The addition of tannin sorghum bran significantly reduced the EGI value of wheat bread, besides being a natural source of brown color, and an excellent source of antioxidants and dietary fiber.

## DEDICATION

I want to dedicate this thesis to my husband, Alejandro. This whole experience was wonderful because you were by my side. Thank you for your encouragement and great example. I love you.

I also dedicate this work to my parents, Cristóbal and Angelina, and my brothers, Cristóbal and Fernando. Thank you for your unconditional love and trust; you are always in my heart. Finally, this thesis is also dedicated to my family-in-law: Enrique A., Lolina, Francis, Enrique, Judith, Federico, María and Marijose. Thanks to all of you for believing in me and all your support.

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# CHAPTER I
# INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is an important source of dietary energy and a main food staple in semi-arid regions of Africa and Asia (Ezeogu et al. 2005). Due to its drought tolerance and adaptation attributes, this grain can be grown in those areas where agricultural and environmental conditions are unfavorable for the production of other crops. Sorghum is considered the world's fifth most important cereal after wheat, rice, maize, and barley (Serna-Saldivar and Rooney 1995). Worldwide, over 35% of sorghum is grown directly for human consumption, while the rest is used primarily for animal feed, alcohol, and industrial products (Rooney and Awika 2004).

In the United States, sorghum utilization directly for human foods is very limited, but with potential to increase (e.g. white food-type sorghum flour has been introduced in gluten-free products such as breads and cookies). Moreover, some specialty sorghums high in tannins have recently shown high antioxidant activities comparable to those of high-antioxidant fruits like blueberries and plums (Awika et al. 2003); giving sorghum an opportunity in functional food markets.

Sorghum generally has the lowest starch digestibility compared to other cereals (Zhang and Hamaker 1998). The lower digestibility of cooked sorghum starch has been shown to affect the feeding value in livestock (Rowe et al. 1999), and to cause a higher loss of energy in humans (MacLean et al. 1981). Sorghum endosperm proteins play an important role in restricting the availability of starch (Rooney and Pflugfelder 1986).

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This thesis follows the style and format of Cereal Chemistry.

Sorghum has extensive genetic variability between varieties, which is responsible for the considerable variation in the nutritional value of sorghum (Lamar 1973). According to Back Knudsen et al. (1988), low-polyphenol varieties are expected to be highly digestible. On the other hand, polyphenols present in several sorghum varieties (e.g. tannin sorghums) are known to alter digestion and absorption processes in simple-stomach animals (Waniska and Rooney 2000). However, Elkin et al. (1996) showed that tannins are only partially responsible for the variation in protein digestibility of sorghum grain cultivars. Therefore, other components also cause the differences in the availability of nutrients in sorghum. Among the factors that influence starch digestibility of cereals are the botanical source, food processing, gelatinization characteristics, particle size, amylose/amylopectin ratio and presence of lipid-amylose complexes (Frei et al. 2003).

The glycemic index (GI) is a concept introduced by Jenkins et al. (1981) to classify foods based on their immediate effect on blood glucose levels. The GI is defined as the postprandial incremental glycemic area after a test meal, expressed as the percentage of the corresponding area after an equi-carbohydrate portion of a reference food (glucose or white bread). From a nutritional point of view, a low glycemic response is considered beneficial for prevention of common diseases such as coronary heart disease, diabetes and obesity. Therefore, there has been a recent interest in slow digesting starches due to their beneficial role in human health.

Sorghum may possibly be considered an alternative to other fast digesting cereal sources, however there is very little, if any, available information on the glycemic index of sorghum-based food products. It is necessary to determine if it would be possible to take advantage of the nutritional characteristics of sorghum in the design of low digestible foods. This information could give sorghum the potential for use in whole-grain products to maintain health and prevent disease.

The objectives of this research were to:

1) Determine the in-vitro starch digestibility rate, and estimated glycemic index of sorghum products prepared with different grain types and fractions.
2) Determine if grain type, grain fraction and preparation treatment, have a significant effect on the estimated glycemic index of the products.

# CHAPTER II
# LITERATURE REVIEW

## Sorghum

Sorghum is a grain rich in starch (≥70% with approximately 75:25 amylopectin/amylose ratio) and it is primarily used in diets as an energy source (Ezeogu et al. 2004). Sorghum kernels are generally spherical and have 1,000-kernel weight ranging from 20 to 30 g, and may be red, white, yellow or brown (Hoseney et al. 1981). Proximate grain composition is 7.9% pericarp, 9.8% germ, and 82.3% endosperm, and unlike other cereals, many sorghum varieties contain starch granules in the pericarp (Hoseney 1994).

The mature sorghum caryopsis may or may not have a pigmented inner integument in the inside-most layer of the pericarp, often called a pigmented testa. When the testa is present, it contains condensed tannins (Waniska and Rooney 2000). Sorghum can be classified based on extractable tannin content (Earp et al. 2004). Type I sorghums have low phenol levels and no pigmented testa or tannins. Both type II and III sorghums have tannins and pigmented testas. However, type II sorghums (containing recessive S gene), have tannins that are extracted by acidic methanol, while tannins from type III sorghums (containing dominant S gene) are extracted by methanol or acidic methanol (Hahn et al. 1986).

Several studies have shown that consumption of high tannin sorghum reduces weight gain of animals (Mariscal-Landín et al. 2004; Muriu et al. 2002; Jambubathan and Mertiz 1973). This is explained by the tannins ability of binding proteins and carbohydrates into insoluble complexes that cannot be broken down by digestive enzymes (Hagerman and Butler 1981). However, epidemiological data on humans in this area is lacking because obesity is not a problem in the places where tannin sorghums are consumed due to different lifestyles, daily food intake, and other factors (Awika and Rooney 2004).

**Sorghum based food products**

Sorghum is the staple food crop in many semi-arid regions of Asia, Africa, and Central America where it is used in traditional foods such as porridges, thick beers, flat breads, and couscous (Acosta 2003).

Grain properties required to obtain acceptable quality food products have been studied. According to Rooney and Waniska (2000), the factor that most consistently affects the processing and food-making properties of sorghum is the endosperm texture (i.e. relative proportion of vitreous to floury endosperm). When decorticating a hard grain, fewer kernels are broken compared to a grain with a floury endosperm texture. During grinding, the vitreous endosperm portion of the grain gives rise to larger flour particles, whereas the floury endosperm forms smaller flour particles.

With regard to cooking quality, studies have shown that grains with a relatively higher proportion of vitreous endosperm are preferred to make thick porridges and for popping (Cagampang et al. 1982, Chandrashekar and Desikachar 1986), while sorghums with high proportion of floury endosperm are preferred for fermented or unfermented breads (Rooney et al. 1986). Hard grains take up less water during cooking, giving a less sticky porridge than soft grains. The stickiness of the cooked sorghum flour is a function of starch gelatinization (Hoseney 1986). The prolamin proteins in sorghum (kafirins) located in protein bodies, have been suggested to be responsible for the functional differences between vitreous and floury endosperm sorghums during cooking (Chandrashekar and Kirelis 1988).

**Starch in sorghum**

Starch is the storage polysaccharide of cereal grains, and compromises generally between 60 and 75% of the weight of the grains (Hoseney 1994). Starch is mainly found in the endosperm, in the form of semicrystalline granules embedded in a continuous protein matrix (Rooney and Pflugfelder 1986).

Starch is composed of two types of polysaccharides: amylose and amylopectin. Both are polymers of α-D-glucose connected by (1→4) linkages. Amylose is a linear polymer of α-1,4 linked glucose units. Amylopectin is a much larger extensively branched polymer of linear chains of α-1,4 linked glucose units, with α-1,6 branch points every 20 to 25 glucose residues. A normal sorghum starch granule contains 23-30% of amylose (Waniska and Rooney 2000), and the rest of amylopectin.

Sorghum starch granules are polygonal or spherical and range from 4 to 24 mm (Hoseney et al. 1981). They exist in a highly organized form inside the granules, where amylose and amylopectin are held together by hydrogen bonds. Hard, corneous-type endosperm contains starch granules that are tightly bound in a rigid protein matrix. On the other hand, floury soft endosperm has starch granules that are loosely spaced and loosely surrounded by protein bodies (Turner 2003).

***Starch gelatinization***

Starch gelatinization is the irreversible loss of the native structure of starch when sufficient moisture and energy (mechanical, thermal or chemical) break the intermolecular hydrogen bonds in the crystalline areas. During gelatinization, the starch granules absorb water, swell, and amylose leaches from the granule (Rooney and Pflugfelder 1986). In the case of sorghum, the gelatinization temperature range is 68-78 °C (Hoseney 1994).

Starch in raw grain may be degraded by enzyme action, however, when the starch is gelatinized or disrupted, it is more rapidly degraded (McNeill et al. 1975). A study showed that native and gelatinized starch from sorghum had amylase digestibility of 55-60% and 70%, respectively (Moorthy 2004).

Food processing usually involves moisture, heat and mechanical action which permits gelatinization to occur. In general, when the processing leads to full gelatinization, the starch digestibility is enhanced. For the preparation of traditional stiff porridges, such as “Ugali” or “Tộ”, sorghum flour is stirred into

boiling water, constantly stirring until a stiff paste is formed (Rooney and Waniska 2000). The porridge consists of a matrix of mainly gelatinized starch granules. The extrusion process involves mixing, shearing and heating in a very short period. The materials flow through a barrel with a screw that shears, heats, and kneads the mass into a continuous melt that is forced into a die where the product forms and expands (Rossen and Miller 1973). Starch is fully gelatinized and protein denatured, and depending on the severity of the process, fragmentation and formation of complexes may follow gelatinization of starch (Acosta 2003).

In bread making, the dry ingredients are mixed with water to form a dough that is further baked at high temperature (210°C) for around 20 minutes. In the crumb of the bread, complete starch gelatinization occurs (Lang 2004), while in the crust incomplete gelatinization takes place due to water evaporation on the surface of the bread during baking.

***Starch hydrolysis***

Amylases are the enzymes that hydrolyze starch during digestion of starch-containing foods. In the gastrointestinal tract, this is achieved by the action of α-amylases and α-glucosidases (Butler et al. 2004). Amylases of major importance are shown in Table 1. The α-amylases randomly hydrolyze α-1,4 glucosidic bonds, generating maltose and short linear oligosaccharides. β-amylases and amyloglucosidases (glucoamylases) have exo-amylase activity, attacking the terminal glucose residues to yield maltose and glucose, respectively. Other enzymes, such as pullulanases, are classified as debranching enzymes, which may also be used in some starch digestibility systems.

**Table 1. Starch hydrolyzing enzymes.**

| Enzymes | Bond(s) hydrolyzed | Products |
|---|---|---|
| α-amylases | Endo- α -1,4 | Maltose + dextrins |
| β-amylases | Exo- α -1,4 | Maltose |
| Amyloglucosidases | Exo- α -1,4 and α -1,6 | Glucose |
| Pullulanases | Endo- α -1,6 | Linear dextrins |
| Isoamylases | Endo- α -1,6 | Linear dextrins |

The rate of starch hydrolysis can be estimated in vitro. Granfeldt et al. (1992) developed a method to measure starch digestion after the food has been chewed under standardized conditions and digested with pepsin. Incubation is done using α-amylase in a dialysis tube, where samples are taken at different time intervals to calculate the degree of hydrolysis.

***Factors affecting starch digestibility***

The degree of starch gelatinization is not the only factor that affects susceptibility of starch to enzyme action (McNeill et al. 1975). The digestibility of starch can be affected by several factors including the composition and physical form of the starch, protein-starch interactions, the physical form of the food, the integrity of the starch-containing cells, and presence of antinutritional factors.

In sorghum, interaction with proteins seems to reduce the susceptibility of both native and processed starch to enzyme hydrolysis because starch granules can be completely embedded in a protein matrix in the corneous endosperm (Rooney and Pflugfelder 1986). According to McNeill et al. (1975), processing methods that alter the kernel structure by releasing the starch granules from the protein matrix will enhance their susceptibility to enzyme action and subsequent digestibility. It is also possible for gelatinized starches to form complexes with proteins, reducing the digestion of both (starch and protein). In addition, antinutritional factors such as tannins present in some sorghums, have been

shown to bind to proteins and reduce starch digestion by inhibiting some enzyme systems (Waniska and Rooney 2000).

**Starch digestibility and health**

General recommendations for carbohydrate sources are to consume whole-grain cereal products and products rich in dietary fiber (Dietary Guidelines for Americans, Anonymous 2005). Among the health benefits from whole-grain/high-fiber foods is their slower digestion and absorption compared to refined products.

Dietary carbohydrates can be classified in many different ways; simple or complex, sugars or starches, available or unavailable. However, besides the classification on the basis of their chemical characteristics, the FAO/WHO has classified carbohydrates considering their physiological properties. Therefore, carbohydrates can be classified according to their potential impact on blood glucose, which can be measured as a "glycemic response" or glycemic index.

For nutritional purposes, Englyst et al. (1992) proposed a classification of starch based on the rate and extent of the starch digestion and developed an in vitro enzymatic method to measure rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). They concluded that the RDS has a large impact in the glycemic response in humans, while the impact from SDS is small. The RS escapes digestion in the small intestine (may be digested in the large intestine), without causing a glycemic response.

***Glycemic index***

The Glycemic Index (GI) is a ranking of carbohydrates based on their immediate effect on blood glucose (blood sugar) levels. As a result, the GI can be a useful indicator of starch digestion of food-based products. WHO/FAO define GI as the incremental area under the blood glucose response curve of a 50 g available carbohydrate portion of a test food, expressed as a percent of the response to the same amount of carbohydrate from a standard food consumed by the same subject. Even though the carbohydrate portion measured is

available or digestible, the GI measures the impact of all carbohydrates on blood glucose levels without correcting for available vs. non-digestible carbohydrates (e.g. resistant starch, dietary fiber).

During digestion, carbohydrates that break down quickly have high glycemic indexes (giving fast and high glucose responses). On the other hand, carbohydrates that break down slowly have low glycemic indexes (releasing glucose gradually into the blood stream). Lowering postprandial blood glucose (by consuming low GI foods) has positive health outcomes for both healthy subjects and patients with insulin resistance. These effects are summarized by Lang (2004) (Fig. 1).

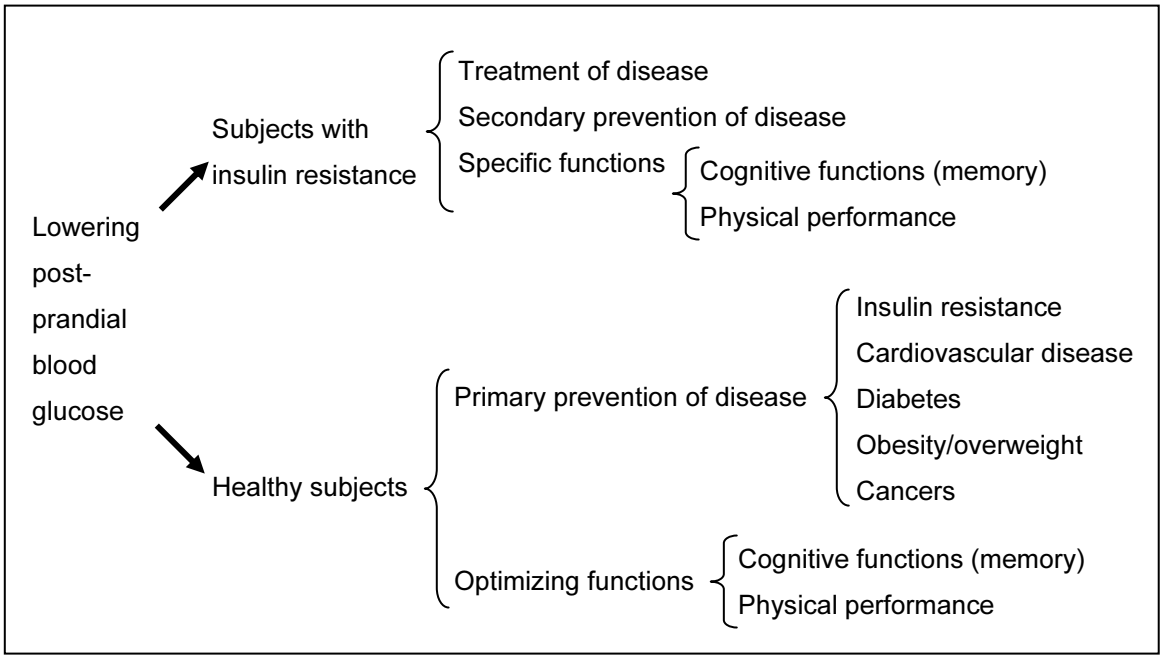


**Fig. 1. Potential beneficial impact of reduced post-prandial blood glucose response on various body functions (Adapted from Lang 2004).**

By definition, GI is measured in vivo. Therefore, the GI is not only influenced by the type of carbohydrates ingested, but also by food-mediated effects on both gastrointestinal events and postabsorptive metabolism (Englyst and Englyst 2004). For instance, some factors that can affect gastric emptying are food particle size, fat content, and viscous fibers (Jenkins et al. 1981).

***Measuring glycemic index in vitro***

Glycemic index evaluation in humans can be difficult and costly, therefore studies measuring in vitro digestion of starch foods have been done in order to predict in vivo effects (Grandfelt et al. 1992, Englyst and Hudson 1996, Björck 1996, Goñi et al. 1997). While Englyst and Hudson (1996) proposed the utilization of rapidly digestible starch expressed in foods as eaten, Goñi et al. (1997) developed a first order equation from the in vitro kinetics of starch digestion of foods. This model has a high correlation with in vivo glycemic responses ($r=0.909$, $p<=0.05$), in addition to good reproducibility and application in other studies (Table 2).

**Table 2. Recent studies using the Goñi et al. (1997) model.**

| Foods | Authors |
|---|---|
| Sorghum and maize flours | Ezeogu et al. (2005) |
| Raw and processed mucuna beans | Siddhuraju and Becker (2005) |
| Rice differing in amylose contents | Hu et al. (2004) |
| Six rice cultivars | Frei et al. (2003) |
| Starchy foods: polished and whole rice, corn, polenta, white spaghetti, potatoes, peas, beans, lentils, and chickpeas. | Rosin et al. (2002) |

In vitro methods to measure GI can be helpful for the initial screening of grain varieties or for the industrial development of foods. However, in accordance with the definition, the GI must be confirmed in vivo by clinical trials.

# CHAPTER III
# EFFECTS OF SORGHUM TYPE AND FRACTION ON STARCH AVAILABILITY OF EXTRUDATES

## Introduction

Extrusion is a process where materials flow through a barrel with a screw that shears, heats, and kneads the mass into a continuous melt that is forced into a die where the product forms and expands (Rossen and Miller 1973). The products obtained are called extrudates. In the food market, the extrudates are seasoned with sweet or salty toppings (e.g. snacks, breakfast cereals), and are called “puffed” products. In the dry friction-type extruder, the energy input into the materials is obtained from the friction generated inside the extruder; therefore, the degree of starch degradation in the product is related to the characteristic of the raw materials (e.g. particle size, endosperm hardness, moisture).

Sorghum is not a major ingredient in extruded snacks in the United States (Riaz 1997); however, its excellent extrusion performance and product characteristics have been recently documented (Acosta 2003, Turner 2004, Perez 2005). Sorghum can be used as a substitute for rice to produce bland, white extrudates. Presently, this is happening in Japan where white food-type sorghums are used in many snacks.

## Objective

The objective of this study was to determine the effects of sorghum type and fraction on the digestibility rates and estimated glycemic index of sorghum extrudates. Corn meal was used as a reference material.

## Materials and methods

### *Sorghum grain characterization*

The sorghum types used were the white food-type (ATx635*RTx436, College Station, 2003) and the tannin “Sumac” (West Texas, 2003). For control, a commercial refined corn meal (Cargill Inc, Minneapolis, MN) was used. Composition of sorghums was determined by Near Infrared Reflectance (NIR) with a Perten model PDA 7000. Sorghum grains were characterized for hardness, diameter and weight with a single kernel hardness tester (SKHT, model SKCS 4100, Perten Instruments, Reno, NV). Density was measured using a gas-comparison pycnometer (Multipycnometer, Quantachrome, Syosset, NY). Hardness index was determined with a Tangential Abrasive Dehulling Device (TADD) using a 20 g sample and 3.5 min abrasion time. Thousand-kernel weight (TKW) was performed by weighing 100 kernels and multiplying by 10. The color of the grains was measured with a colorimeter (model CR-310, Minolta, Osaka, Japan) using CIE L* a* b* color scale. To identify the presence of pigmented testa, the Clorox bleach test was performed based on the method used by FGIS-GIPSA. Five kernels of each type of sorghum were dissected and endosperm appearance was evaluated visually.

### *Grain preparation*

Clean grains were extruded intact or prepared by two methods: cracking and decortication. Sorghum kernels were cracked using an attrition mill of 1.5 HP (Glen Mills Inc, Marywood, NJ) to obtain halves and quarters. The cracked sample was used for extrusion without sifting to remove the fine particles. Sorghum kernels were decorticated in 4-kg batches in a PRL mini-dehuller (Nutama Machine Co., Saskatoon, Canada) to remove the pericarp fraction. The bran was removed with a KICE grain cleaner (Model 6DT4-1, KICE Industries Inc., Wichita KS).

***Particle size distribution***

Particle size distribution of the grains and fractions was calculated using #10, 20, 30, 40, and 60 US standard sieves and 50 g sample size. Results were reported as percentage retained above each sieve. Measurements for each sample were made in duplicate.

***Extrusion***

Whole, cracked, and decorticated sorghums, and corn meal were tempered to 14% moisture. Distilled water was added to the kernels inside rigid plastic bottles (1 gal) attached to a tumbler rotating at 37 rpm for 4 h. Then the samples were equilibrated in the closed containers at 21°C for 24 h prior to processing.

Tempered samples were added to a single-screw friction-type extruder (Model MX- 300I, Maddox Inc, Dallas, TX). The extruder was pre-heated to 325 ºF and the screw speed was set to 341 rpm. Extrusion conditions (temperature, power consumed, and time) were recorded for each sample. After extrusion, the samples were baked at 100°C for 30 min in a convection oven and they were stored in plastic bags at -20°C until analyzed. The baked extrudates were used to measure starch digestibility. Subsamples were ground in a UDY cyclone mill (Model 3010-030, Udy Corporation, Fort Collins, CO) using a 1.0 mm round-hole screen for the analysis of the starch fractions.

***Moisture***

Moisture content of the extrudates after drying was determined by the moisture air oven method (AACC Method 44-19) in duplicate. Extrudates were not ground.

***Expansion ratio***

Expansion ratio was calculated according to Gomez et al. (1988). The diameter of 25 extrudates was measured with an electronic caliper, and each value was divided by the die-hole diameter (1/8” or 3.175 mm).

***Microscopy***

Selected samples were analyzed by Bright Field Microscopy (Zeiss Universal) using polarized light to look at the degree of starch gelatinization in the products.

***Starch fractions***

*Total starch*

Total starch (TS) was determined by the AACC Method 76.13 using the Total Starch Assay Procedure Kit (Megazyme Int, Ireland). A 100 mg of dried ground sample was dispersed with 0.2 mL of aqueous ethanol (80%v/v). Immediately 3 mL of thermostable α-amylase in a MOPS buffer was added and the tube was incubated in a boiling water bath for 6 min (Stirring the tube vigorously after 2 min and 4 min). The tube was placed in a water bath at 50°C, and 4 mL of sodium acetate buffer (200 mM, pH 4.5) was added followed by amyloglucosidase (0.1 mL, 20 U).The tube was stirred on a vortex mixer and incubated at 50°C for 30 min. Then, the volume was adjusted to 100 mL with distilled water. An aliquot of this solution was centrifuged at 3,000 rpm for 10 min. Duplicate aliquots (0.1 mL) were transferred to test tubes and 3 mL of the glucose oxidase reagent was added. The incubation with the reagent was at 50°C for 20 min, and the absorbance was measured at a wavelength of 510 nm against the reagent blank. Glucose concentration was converted into starch by multiplying by 0.9. Each sample was analyzed in triplicate.

*Resistant starch*

Resistant starch (RS) was determined enzymatically by the method of Goñi et al. (1996). 100 mg of ground sample was incubated with a solution of 20 mg of pepsin from porcine gastric mucosa (P-7000, Sigma-Aldrich Inc.) in a KCl-HCl buffer for 60 min at 40°C. After cooling the sample at room temperature, 9 mL of 0.1 M Tris-maleate buffer (pH 6.9) was added followed by 1 mL of a solution of 40 mg of α –amylase from porcine pancreas (A-3176, Sigma-Aldrich Inc.). The sample was incubated at 37°C for 16 h with constant shaking. The

hydrolyzate was centrifuged and the supernatant discarded. The residue was moistened and 3 mL of KOH was added to solubilize the residual starch, shaking for 30 min at room temperature. After adjusting the pH to 4.75 (using 0.4 M sodium acetate buffer and 2 M HCl), 80 µl of amyloglucosidase from *Aspergillus niger* (A-1602, Sigma-Aldrich Inc.) was added, mixed well and left for 45 min in a water bath at 60°C with constant shaking. The solution was centrifuged and the supernatant collected in a 25 mL volumetric flask. After adjusting the volume with distilled water, duplicate aliquots (0.1 mL) of this solution were transferred into test tubes and the reagent from the glucose determination kit (Megazyme Int, Ireland) was added and the absorbance was read as described in the total starch analysis. The resistant starch was calculated as mg of glucose x 0.9. Samples were analyzed in triplicate.

*Digestible starch*

Digestible starch (DS) was calculated as the difference between TS and RS.

***In vitro rate of starch digestion***

The procedure and model established by Goñi et al. (1997) was used to measure the in vitro starch hydrolysis. Triplicate samples of 50 mg of prepared samples (as ready to eat) were homogenized in water using a Polytron homogenizer (Kinematica GmbH, Switzerland) with controlled speed (level 4, 1 min). Then, 0.2 ml of a solution containing 1 mg of pepsin from porcine gastric mucosa (P-7000, Sigma-Aldrich Inc.) in 10 mL of HCl-KCl buffer pH=1.5 was added. The samples were incubated at 40°C for 60 min in a shaking water bath. 15 ml of Tris-Maleate buffer (pH 6.9) was added to adjust pH. Then another 5 ml of Tris-Maleate buffer containing 2.6 UI of α-amylase from porcine pancreas (A-3176, Sima-Aldrich Inc.) was added. The flasks were placed in a water bath at 37°C with agitation. Aliquots (0.1 ml) were taken every 30 min form 0 to 3 h. α -amylase was inactivated by immediately placing the tubes in a boiling water bath for 10 min with vigorous shaking every 30 sec. Then, 1 ml of 0.4 M sodium-

acetate buffer pH=4.75 and 30 µl of amyloglucosidase from *Aspergillus niger* (A-1602, Sigma-Aldrich Inc.) were added. The samples were incubated at 60°C for 45 min to hydrolyze the digested starch into glucose. Finally, the glucose concentration was measured using the glucose oxidase-peroxidase kit. (Megazyme Int, Ireland) as described previously in the total starch analysis. The experiment was repeated two times for each sample.

The rate of starch digestion was expressed as a percentage of total starch hydrolyzed at different times (30, 60, 90, 120, 150, and 180 min). The digestion curves were adjusted to the following non-linear equation established by Goñi et al. (1997) to describe the kinetics of starch hydrolysis:

$$C = C_\infty (1\text{-}e^{-kt})$$

where $C$ is the percentage of starch hydrolyzed at time $t$ (min), $C_\infty$ is the equilibrium percentage of starch hydrolyzed after 180 min, and k is the kinetic constant. The variables $C_\infty$ and k were estimated for each sample using SPSS for Windows 11.5.

*Rapidly and slowly digested starch*

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were determined according to Englyst et al. (1992). The enzymatic hydrolysis method of Goñi et al. (1997) was used to obtain these fractions. The RDS was defined as the percentage of starch digested at 30 min, and the SDS as the percentage of starch digested at 120 min.

*Hydrolysis index and estimated glycemic index*

From the digestion curves obtained during starch hydrolysis, the area under the hydrolysis curve (AUC) was calculated for each sample using the equation:

$$AUC = C_\infty (t_f - t_o) - (C_\infty/k)[1 - \exp [-k(t_f - t_o]]$$

where $t_f$ is the final time (180 min) and $t_o$ is the initial time (0 min). The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (fresh white bread,

GI=100) obtained from Goñi et al. (1997). Finally, the estimated glycemic index (EGI) was predicted with the formula:

$$EGI = 39.71 + (0.549 \times HI).$$

***Statistical analysis***

Mean values of all data were analyzed with one way analysis of variance (ANOVA) and significant differences were tested by Duncan's test using a confidence level of 95% ($\alpha$=0.05). The statistical software SPSS v 11.5 (SPSS Inc., Chicago, IL) was used.

## Results and discussion

***Sorghum grain characterization***

Both sorghum types were morphologically different with significantly different amounts of protein and starch (Table 3). The white sorghum kernels were significantly larger and harder compared to the tannin sorghum kernels. The white sorghum had a light pericarp (higher L value) and more yellow color (higher b value) while the tannin sorghum had darker pericarp with higher red values. The dissected grains showed a higher proportion of hard endosperm in the white sorghum (Fig. 2), and a higher proportion of floury endosperm in the tannin sorghum (Fig. 3). The high amount of soft endosperm from the tannin sorghum caused a higher percentage of removal in the TADD and a lower Hardness Index from the SKHT compared to the white sorghum.

***Grain preparation and particle size distribution***

The whole and cracked sorghum grains, white and tannin, had 100% yield (Table 4). In the first case, the whole kernels were extruded intact, and in the later, the fines were not removed after cracking. Different yields were obtained when the sorghum grains were decorticated. The white sorghum showed a greater yield compared to the tannin sorghum. Endosperm texture (i.e. relative proportion of vitreous to floury endosperm) was the factor that affected the percentage of decortication. According to Rooney and Waniska (2000), kernels with hard endosperm and thick pericarp are easily decorticated; less

time is needed to decorticate, and better yields are obtained. On the other hand, sorghums with soft, floury kernels break into pieces and the pericarp cannot be separated without sacrificing yields.

Fig. 4 shows the final appearance of the grains prepared for extrusion. The decorticated tannin sorghum reveals that after removing 18% of the kernel weight in the decortication process, the pericarp was not efficiently removed. For both types of sorghum, the whole kernels had particle sizes above 2000 μm (US sieve #10) (Table 5). Decortication of the white sorghum was very efficient (the kernels did not break). On the other hand, decortication of the tannin sorghums generated broken kernels/fines (above sieves #20 and #40). Cracked kernels for both types of sorghum had small particles (fines) generated during cracking. Soft tannin sorghum kernels broke into a greater proportion of smaller pieces.

***Extrusion***

Extrudates had acceptable appearance (Fig. 5), characteristic of “puffed” snacks or breakfast cereals. White sorghum extrudates were more expanded than the tannin sorghum extrudates for all preparation methods. The tannin extrudates had a brownish color due to the pigments naturally present in the pericarp and testa of these kernels. Some of the pericarp remained in the decorticated tannin kernels, and the extrudates of this grain fraction had a lighter brownish color.

Extrusion parameters are shown in Table 6. SME (energy per unit weight that the material receives inside the extruder) and power consumed were similar for all the sorghum treatments, but higher for the corn meal. According to Turner (2004), when the SME is increased, the degree of starch gelatinization is also increased. Any non-starch materials, such as fiber, lipids, or protein, will decrease SME when the rest of the extrusion factors are constant. In this case, the SME of the corn meal was followed by the decorticated sorghums. On the other hand, whole grain materials (with higher amounts of fiber and oil) reduced

**Table 3. Physical characteristics of the white and tannin sorghums.**

| | White sorghum* | Tannin sorghum |
|---|---|---|
| Protein (% d.b.) | 11.5 a | 12.1 b |
| Starch (% d.b.) | 72.5 a | 68.4 b |
| Hardness index (SKHT) | 93.4 a | 57.9 b |
| Weight (mg) | 29.8 a | 16.8 b |
| Diameter (mm) | 2.5 a | 1.9 b |
| TADD (% weight removed) | 11.6 a | 20.6 b |
| Density ($g/cm^3$) | 1.3 a | 1.3 a |
| Thousand kernel weight (g) | 30.2 a | 15.5 b |
| Test weight (lb/bu) | 60.1 a | 59.1 b |
| Clorox bleach test | Negative (tannin free) | Positive |
| Color | | |
| L* | 60.4 a | 38.2 b |
| a* | 3.9 a | 9.7 b |
| b* | 18.6 a | 10.0 b |
| Tannins (mg CE/g d. m.r) ** | Trace, zero | 13.7 |
| Phenols (mg GAE/g d.m.)** | 0.8 a | 19.8 b |

* Values followed by the same letter within a row are not significantly different ($p<0.05$).
** From Awika (2003).

**Fig. 2. White sorghum kernels. Intact (top) and dissected (bottom).**

**Fig. 3. Tannin sorghum kernels. Intact (top) and dissected (bottom).**

**Table 4. Yields of sorghum grains used for extrusion.**

| Sample | % Yield |
|---|---|
| Whole white sorghum | 100 |
| Cracked white sorghum | 100 |
| Decorticated white sorghum | 91 |
| Whole tannin sorghum | 100 |
| Cracked tannin sorghum | 100 |
| Decorticated tannin sorghum | 82 |

**Table 5. Particle size distribution (% weight) of raw materials used for extrusion.**

| Grain preparation | Sieve US#10 (2000 µm)* | Sieve US#20 (850 µm) | Sieve US#30 (600 µm) | Sieve US#40 (425 µm) | Sieve US#60 (250 µm) | Pan (<250µm) |
|---|---|---|---|---|---|---|
| Whole white | 99.9 a | 0.1 c | 0.0 b | 0.0 b | 0.0 b | 0.0 b |
| Cracked white | 75.1 b | 23.4 b | 0.2 b | 0.2 b | 0.2 b | 0.2 b |
| Decort. white | 99.7 a | 0.1 c | 0.1 b | 0.0 b | 0.0 b | 0.0 b |
| Whole tannin | 99.9 a | 0.1 c | 0.0 b | 0.0 b | 0.0 b | 0.0 b |
| Cracked tannin | 16.5 c | 75.3 a | 3.1 a | 2.3 a | 1.7 a | 0.9 a |
| Decort. tannin | 95.3 a | 4.5 c | 0.2 b | 0.0 b | 0.0 b | 0.0 b |
| LSD | 5.8 | 4.7 | 0.3 | 0.2 | 0.2 | 0.4 |

* Treatments with the same letter within each column are not significantly different ($p<0.05$).

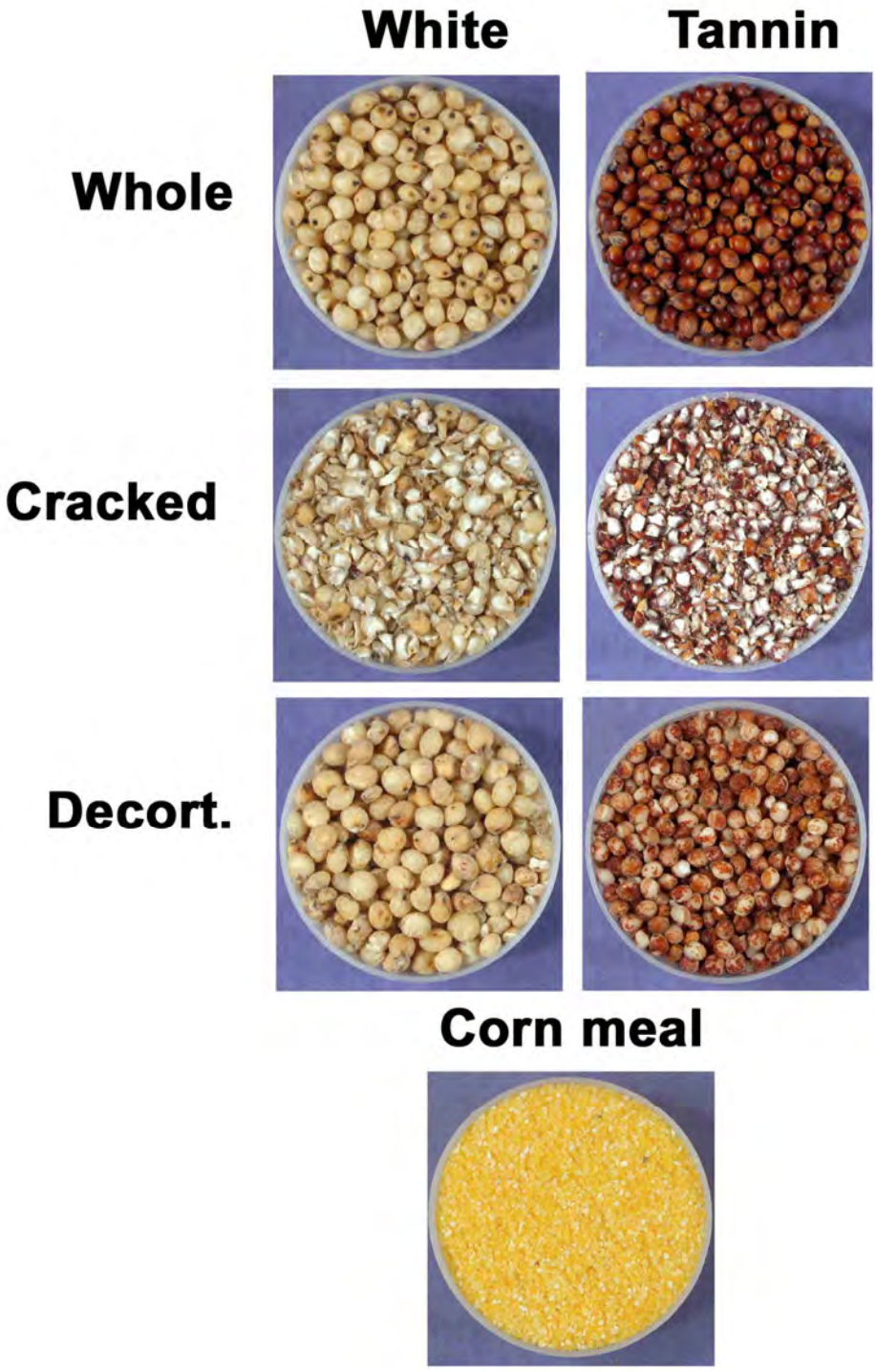


**Fig 4. Raw materials used for extrusion.**

the SME, and therefore, the starch was less transformed inside the extruder.

***Expansion ratio***

The expansion ratio of the extrudates was affected by the type of grain and fraction used (Fig. 6). Corn meal extrudates had the greatest expansion, followed by the decorticated white and tannin extrudates. The whole grain extrudates (from grains processed as intact or cracked) had the least expansion for both sorghums. According to Horn (1977), starch is the primary component on which expansion depends. Corn meal is highly refined, composed primarily of starch (e.g. lower ash, fiber, oil), giving maximum expansion and gelatinization. As expected, decortication of sorghum increased the expansion, but still decorticated sorghum extrudates were not as expanded as corn meal extrudates. This could be due to the differences in particle size. Acosta (2003) showed that decreasing the particle size of decorticated sorghum (using a sorghum meal) caused increased expansion upon extrusion.

***Microscopy***

Extrudates from corn meal, decorticated white, and decorticated tannin sorghum were analyzed with light microscopy. A gelatinized melted matrix was apparent in corn meal extrudates (Fig. 7, A). Corn meal developed the highest SME inside the extruder, therefore, high viscosity was developed, allowing a complete starch gelatinization and dispersion (Fig. 7, A). The decorticated white extrudate had increased gelatinization, however it shows a melted matrix with some intact, non-dispersed gelatinized granules (Fig. 7, B). This is related to a lower SME and power input in the white sorghum compared to the corn meal during extrusion (Table 6). Finally, the decorticated tannin sorghum extrudate shows intact starch granules that are fully gelatinized but not dispersed in a melted matrix (Fig. 7, C). The extrusion parameters show a significant reduction in SME, power, and temperature when this sample was extruded, suggesting that the soft nature of the grain did not permit enough friction and viscosity development to completely degrade the starch in the sample.

**Table 6. Extrusion parameters.**

| Grain preparation | Temperature (F) | Power (kW) | Feed rate (kg/h) | SME (kJ/kg) |
|---|---|---|---|---|
| Corn meal | 318 | 23.6 | 161.2 | 526.9 |
| Whole white | 328 | 18.0 | 189.5 | 341.3 |
| Cracked white | 323 | 17.5 | 166.2 | 380.2 |
| Decort. white | 318 | 21.9 | 189.5 | 415.6 |
| Whole tannin | 335 | 17.8 | 168.8 | 380.6 |
| Cracked tannin | 327 | 15.9 | 161.2 | 354.8 |
| Decort. tannin | 299 | 18.0 | 174.2 | 372.4 |

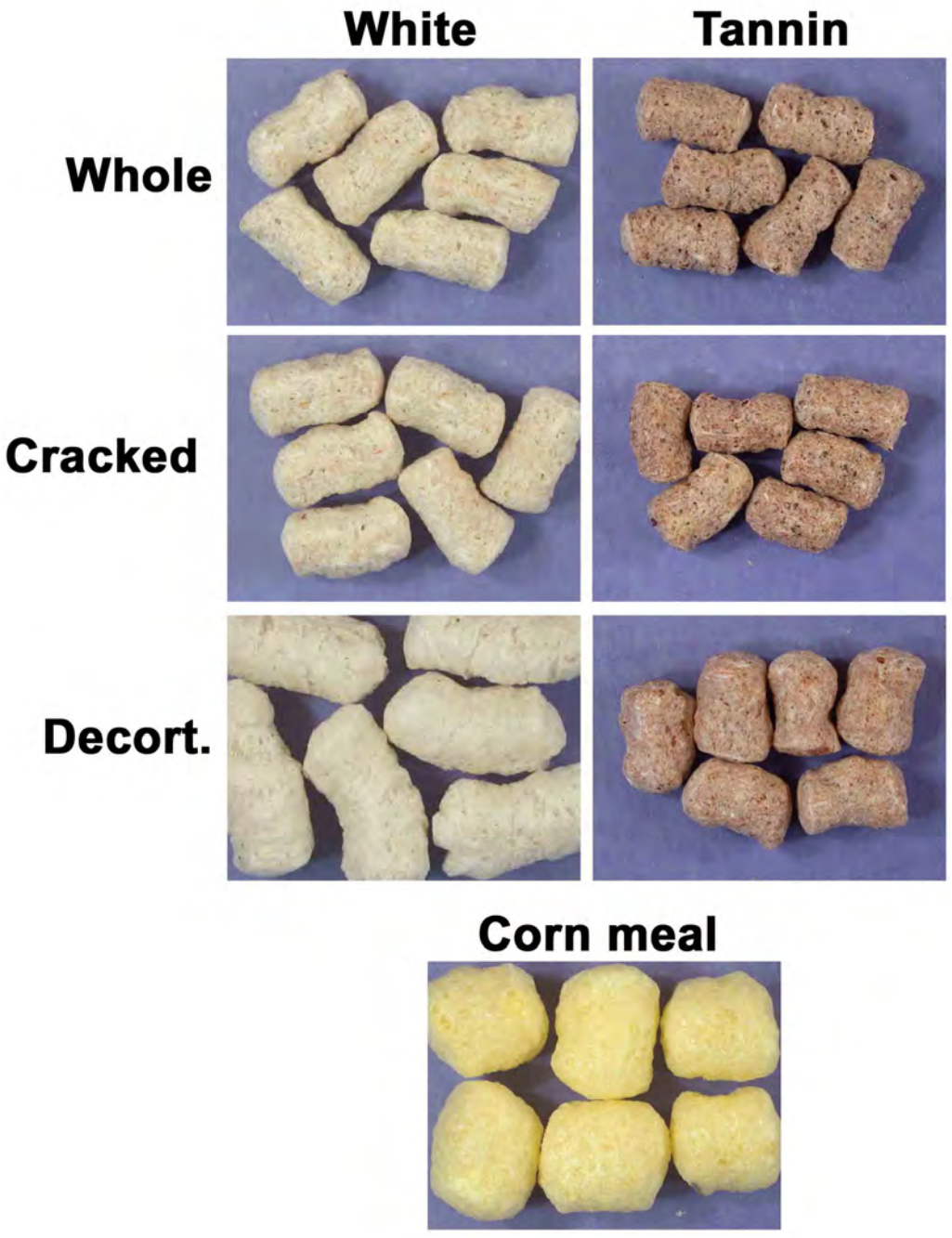


**Fig. 5. Extrudates obtained from the raw materials for extrusion.**

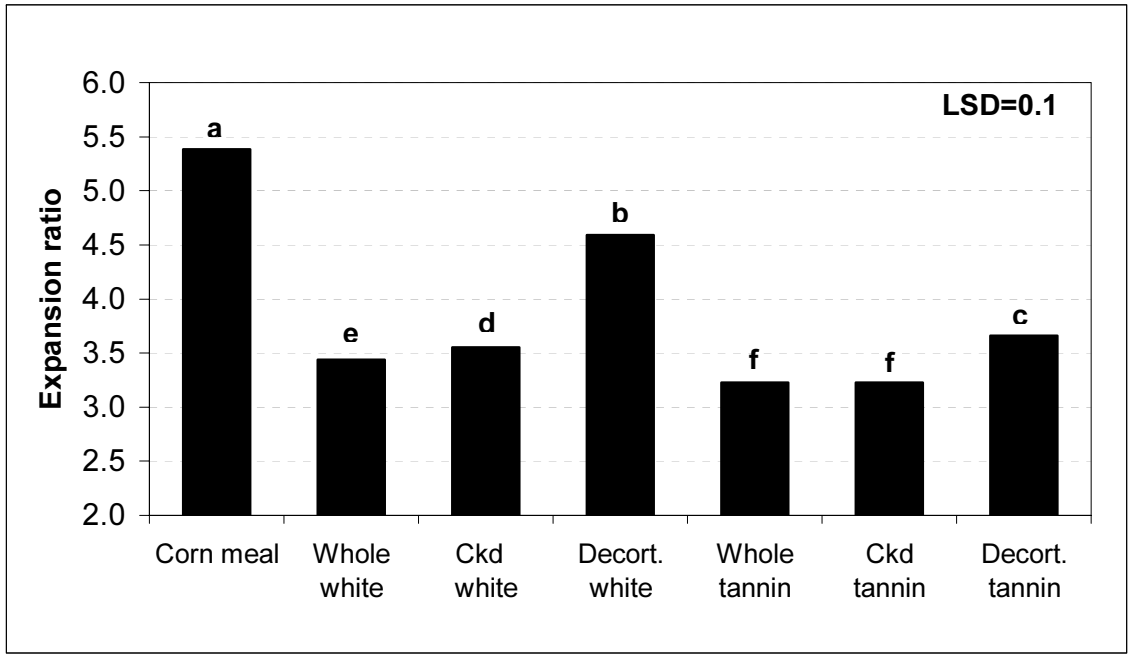


**Fig. 6. Expansion ratio of extrudates. Treatments with the same letter are not significantly different ($p<0.05$).**

***Starch fractions***

Total starch (TS), resistant starch (RS) and digestible starch (DS) fractions are shown in Table 7. Corn meal extrudate had the highest amount of TS among all the samples since it was a highly refined product. For both sorghums, white and tannin, decorticated extrudates had higher amounts of starch compared to the whole extrudates (both whole and cracked) due to the increased concentration of starch by decortication. The whole tannin products had significantly lower amounts of starch compared to the rest of the samples.

According to Goñi et al. (1996), breakfast cereals have low (1-2.5%) to intermediate (2.5-5.0%) RS content depending on the process and processing conditions used. During extrusion cooking, high-temperature and high-shear forces cause a high degree of starch gelatinization, increasing the susceptibility of starch to enzyme hydrolysis (Holm et al. 1985), therefore, low levels of RS were expected. In agreement with the literature, the RS of the extrudates was generally low, and the starch fraction considered digestible was high for all the samples. The samples were processed under similar conditions; however, the RS values were different among most of the samples. For both types of sorghums, the cracked samples showed the highest amounts of RS, suggesting that the small particle size pieces generated during cracking (fines) have a role in increasing this fraction.

According to Rosin et al. (2002), other factors that explain the differences in RS quantities besides the degree or starch gelatinization and particle size, are the type of cellular structure, and the presence of other components such as dietary fiber and antinutrients The tannin sorghum extrudates had the highest amount of RS for all preparation methods compared to the white sorghums (Table 7). The condensed tannins might have associated with other components (such as proteins) which may have interfere with sorghum starch gelatinization (McNeill et al. 1975). Furthermore, the indigestible starch measured by the RS assay is most likely physically inaccessible granules (endosperm pieces) that

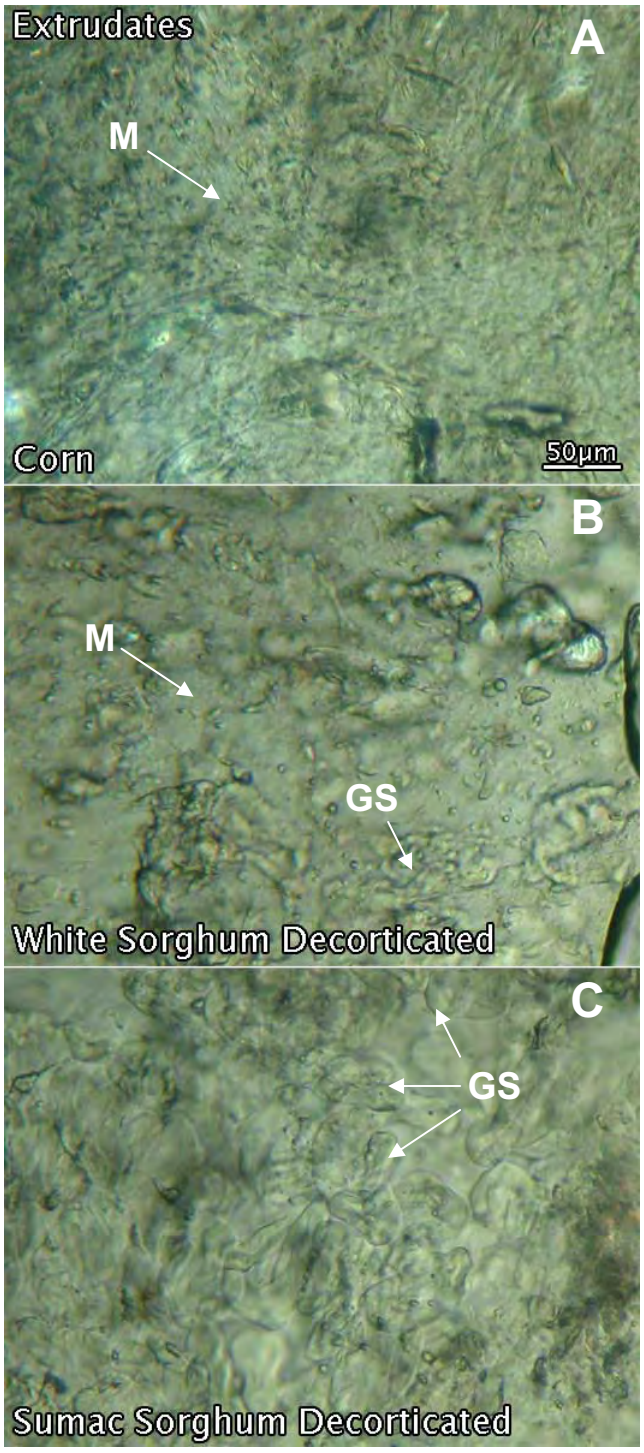


**Fig. 7. Selected extrudates seen under light microscope. A: Corn meal, B: Decorticated white sorghum, C: Decorticated tannin sorghum. M: Gelatinized melted matrix, GS: gelatinized starch granules.**

**Table 7. Moisture and starch fractions of extrudates (% dry weight basis).**

| Type of extrudate | % Moisture* | Total starch | Resistant starch | Digestible starch |
|---|---|---|---|---|
| Corn meal | 1.9 e | 89.4 a | 0.3 f | 89.1 a |
| Whole white | 2.6 c | 76.4 c | 0.4 e | 76.0 c |
| Cracked white | 3.0 b | 77.1 c | 1.3 d | 75.8 c |
| Decort. white | 2.0 e | 80.3 b | 0.2 f | 80.1 b |
| Whole tannin | 2.4 d | 70.0 d | 1.7 b | 68.3 d |
| Cracked tannin | 3.5 a | 68.8 d | 2.6 a | 66.2 e |
| Decort. tannin | 3.0 b | 77.2 c | 1.5 c | 75.7 c |
| LSD | 0.1 | 1.2 | 0.1 | 1.3 |

* Moisture of extrudates after drying.

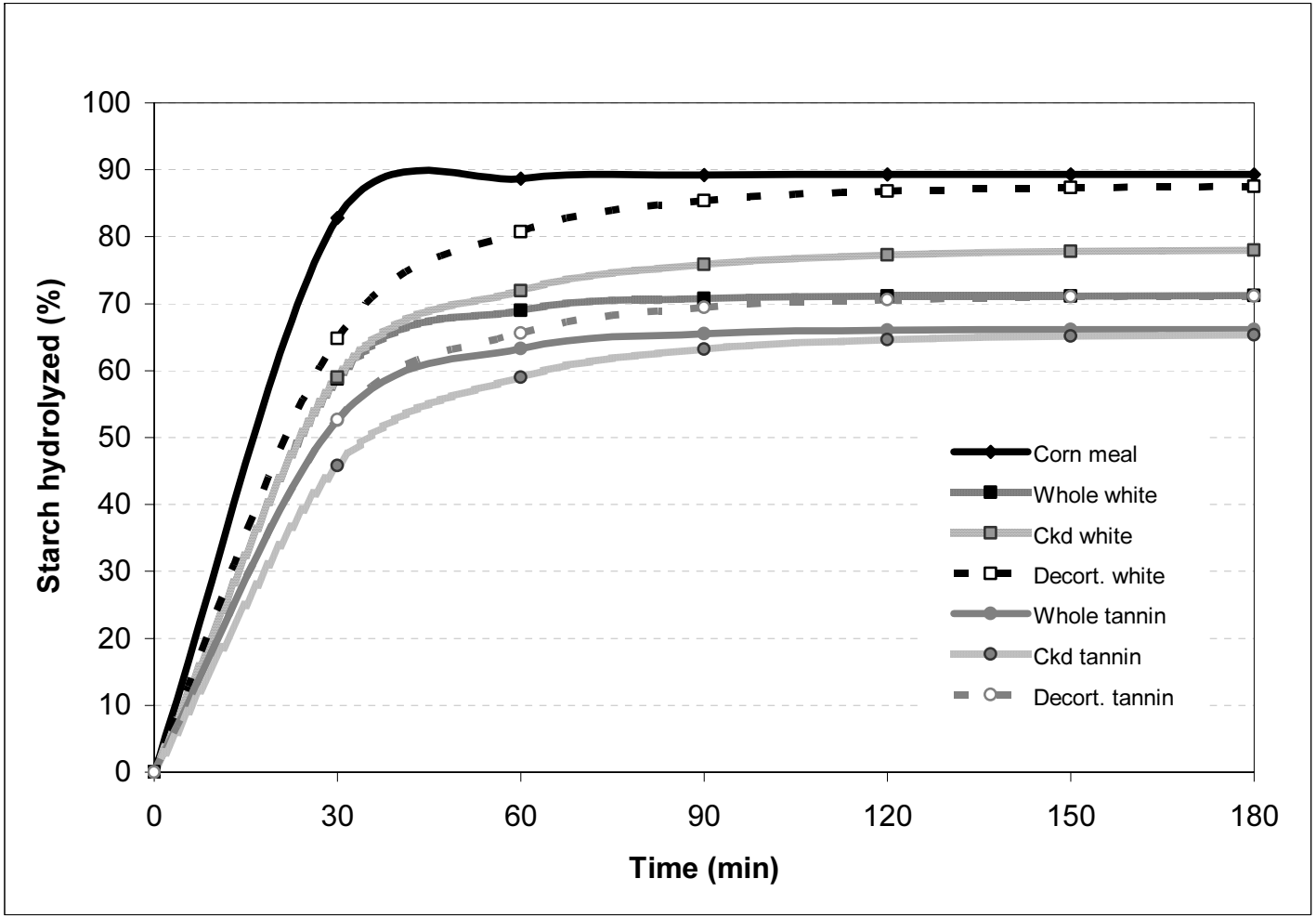


**Fig. 8. In vitro starch digestibility of extrudates from 0 to 180 minutes.**

**Table 8. Percentage (dry wt basis) of starch hydrolyzed at different times (min), and calculated C∞ and k constants for each type of extrudate. Treatments with the same letter within each column are not significantly different (p<0.05).**

| Type of extrudate | Time (min) | | | | | | C∞ | k |
|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | | |
| Corn meal | 82.8 a | 88.7 a | 89.3 a | 89.3 a | 89.3 a | 89.3 a | 89.3 a | 0.092 a |
| Whole white | 58.7 b,c | 69.0 d | 70.8 d | 71.1 c | 71.1 c | 71.2 c | 71.2 c | 0.058 b |
| Cracked white | 59.0 b,c | 71.9 c | 75.8 c | 77.3 b | 77.8 b | 77.9 b | 78.1 b | 0.057 b |
| Decort. white | 64.8 b | 80.7 b | 85.3 b | 86.8 a | 87.4 a | 87.5 a | 87.7 a | 0.048 b |
| Whole tannin | 52.6 c | 63.3 e | 65.5 e | 66.0 d | 66.1 d | 66.1 d | 66.1 d | 0.055 b |
| Cracked tannin | 45.8 d | 59.0 f | 63.2 e | 64.6 d | 65.1 d | 65.3 d | 65.5 d | 0.040 b |
| Decort. tannin | 52.6 c | 65.6 e | 69.4 d | 70.6 c | 71.0 c | 71.2 c | 71.2 c | 0.050 b |
| LSD | 6.3 | 2.6 | 2.8 | 3.5 | 3.8 | 4.0 | 4.1 | 0.0 |

were not fully gelatinized due to insufficient friction and heat generated inside the extruder.

***In vitro rate of starch digestion***

All sorghum extrudates showed a slower rate of starch digestion compared to the corn meal extrudates; meaning that at all sampling times, lower percentages of starch were hydrolyzed in the sorghum products compared to the corn meal sample (Fig. 8, Table 8). However, at 120 min, the decorticated white sorghum extrudate reached the same percentage of starch hydrolyzed as the corn meal. This means that sorghum starch in the decorticated (refined) extrudate took longer to digest than the (refined) corn meal, but resulted in the same percentage of starch being digested after 3 hours.

Decortication increased starch digestibility for each type of sorghum. In a study by Bach Knudsen et al. (1988), decreased starch digestibility of foods prepared from sorghums was explained by a high content of dietary fiber. Our work confirmed that extrudates with higher amounts of fiber (i.e. whole-grain) were less digestible. However, tannin sorghum extrudates were less digestible than white extrudates for all comparisons (Fig. 8). This clearly indicates that tannins produce more slowly digested starch complexes upon extrusion.

The kinetic parameters that describe the hydrolytic process of starch digestion were obtained (Table 8). $C\infty$ represents the equilibrium concentration, reached after 180 min of hydrolysis and the constant “k” stands for the kinetic (digestibility) constant (i.e. intrinsic susceptibility of the starch in the product to digestion). The kinetic constant from the corn meal extrudates was significantly different than all the sorghum extrudates, suggesting that the differences in digestibilities were due to the innate properties of their starches. On the other hand, both the white and the tannin extrudates gave identical kinetic constants. Therefore, the differences in the digestibility of these two grains were probably due to extrinsic factors (Ezeogu et al. 2005). Condensed tannins from tannin sorghums could reduce the starch digestibility, since extrusion may facilitate

association of condensed tannins with starch and protein reducing the digestion of both (McNeill et al. 1975). Another factor involved could be a reduced degree of starch gelatinization in the tannin extrudates (less expanded, more grainy appearance) compared to the white sorghum extrudates.

*Rapidly and slowly digested starch*

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were calculated from the in vitro starch digestion at 30 and 120 min of enzymatic incubation respectively (Fig. 9). The RDS was high for the corn meal extrudate and significantly lower for all sorghum samples. The SDS was the same for the decorticated white sorghum extrudates and the corn meal extrudates, and again, significantly lower for the rest of the white and tannin sorghum extrudates.

RDS has been used as an alternative method to evaluate starch digestion. When the RDS fraction is high, the starch is considered rapidly digested, giving a high glycemic response in vivo (Rosin et al. 2002). The RSD and SDS of corn meal extrudates were statistically the same, indicating that practically all the starch was completely hydrolyzed in the first 30 min. Corn meal and decorticated white sorghum extrudates had the same SDS (the same percentage of starch was hydrolyzed at 120 min), however, the RDS fractions were statistically different. While the corn meal extrudate had a RDS of 82.8%, the decorticated white sorghum had a RDS of 64.8%.

According to Brown (2004), one of the nutritional benefits of whole grain foods is the fact that physical protection afforded to the starch in the intact grains make the starch more slowly digested or indigestible (physically inaccessible starch or RS1). In agreement with the literature, the whole grain extrudates from white sorghum showed lower RDS compared to the decorticated extrudates, and also slightly higher amounts of RS.

On the other hand, whole and decorticated tannin sorghums had the same RDS (lowest among all the samples); therefore, removing the testa layer through decortication did not improve the initial digestibility. However, after 30

min, a slower rate of digestion occurred for the whole extrudates, with a lower SDS compared to the decorticated extrudates.

There was a high correlation between RDS and expansion ratio ($R^2$=0.87) (Fig. 10), suggesting that the most expanded extrudates had higher degree of starch degradation, and were initially more rapidly digested by the α-amylase.

*Hydrolysis index and estimated glycemic index*

The Hydrolysis index (HI) represents the proportion of starch that is theoretically digestible (under the conditions of the study) (Ezeogu et al. 2005). Using the HI value in the formula by Goñi et al. (1997), the estimated Glycemic Index (EGI) for all products was significantly different (Table 9).

The HI values of the extrudates were highly correlated with RDS ($R^2$=0.93) (Fig. 11). Rosin et al. (2002) also found a positive correlation between HI and RDS suggesting that the RDS expressed as dry weight is an alternative predictor of the digestion of starchy foods. Furthermore, a highly significant positive correlation was found between the HI values of the extrudates and their expansion ratio ($R^2$=0.90) (Fig. 12), indicating that the expansion ratio affects the degree of starch degradation. At the same time, the close relationship found between the expansion ratio and the RDS (Fig. 10) confirms that samples had different degrees of starch degradation during the extrusion process.

Grain type and grain fraction affected the EGI. When organizing the EGI in descendent order (Fig. 13), corn meal extrudates had the highest value (108), followed by the decorticated white (102), whole white (96), cracked white (92), decorticated tannin (90), whole tannin (88) and cracked tannin (85).

When comparing the EGI of the sorghum extrudates to the GI of extruded/puffed products from the literature (Table 10), the whole grain sorghums (both from white and tannin varieties) have potential for development of extrudates with lower GI values.

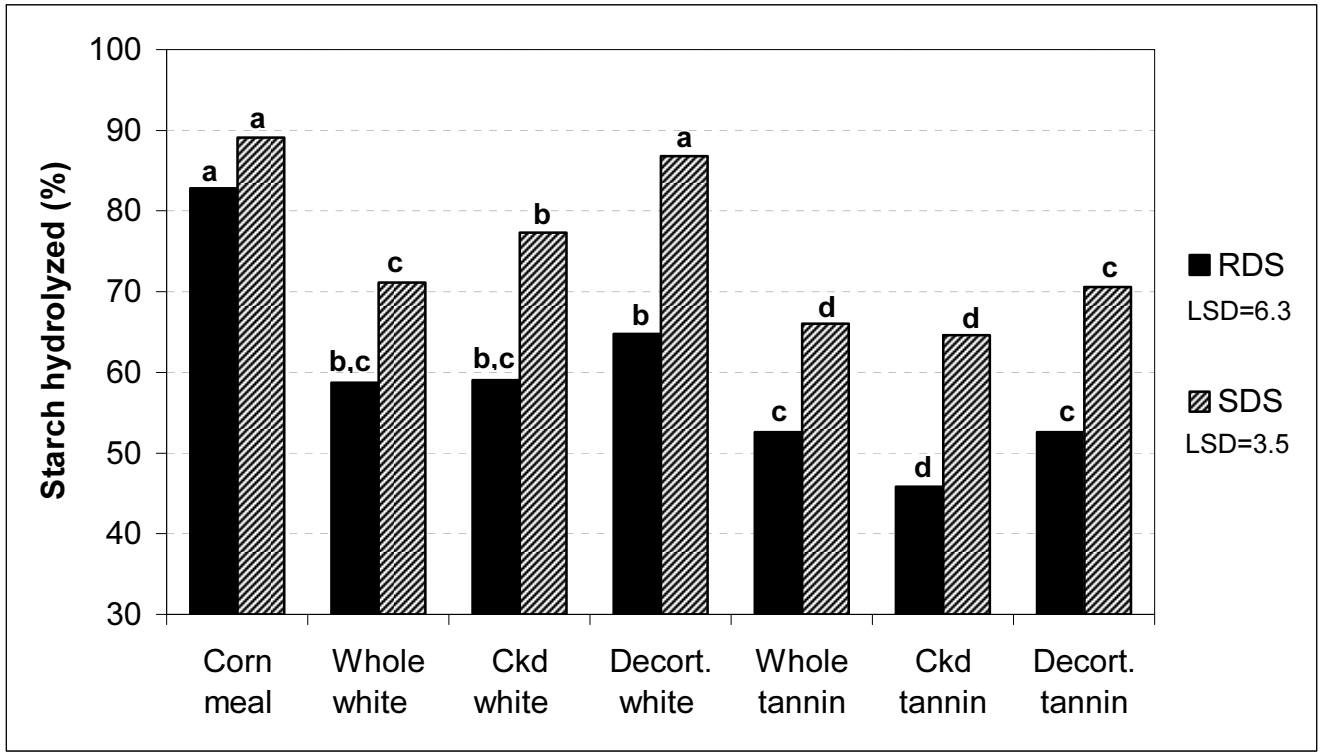


**Fig. 9. Rapidly digestible starch (RDS), and slowly digestible starch (SDS) of extrudates (dry wt basis). Treatments with the same letter within each type of bar are not significantly different ($p<0.05$).**

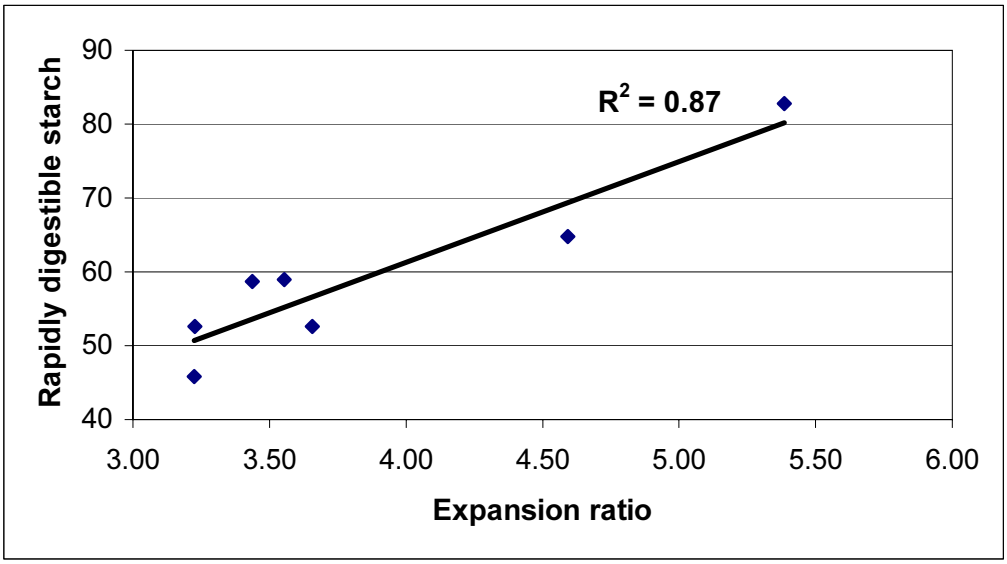


**Fig. 10. Correlation between rapidly digestible starch (dry wt. basis) and expansion ratio of extrudates.**

**Table 9. Hydrolysis Index (HI) and estimated Glycemic Index (EGI) of extrudates.**

| Extrudate | HI | EGI |
|---|---|---|
| Corn meal | 124 ±1 a | 108 ±1 a |
| Whole white | 96 ±2 d | 92 ±1 d |
| Cracked white | 102 ±3 c | 96 ±2 c |
| Decort. white | 113 ±4 b | 102 ±2 b |
| Whole tannin | 88 ±3 f | 88 ±1 f |
| Cracked tannin | 83 ±4 g | 85 ±2 g |
| Decort. tannin | 92 ±2 e | 90 ±1 e |
| LSD | 3.2 | 1.8 |

*: ±SD.

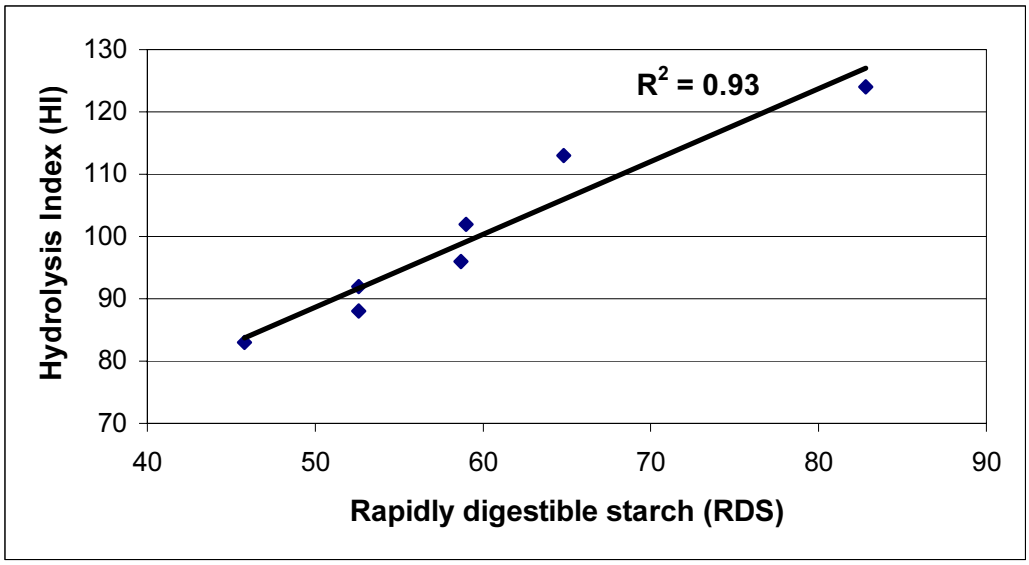


**Fig. 11. Correlation between Hydrolysis Index (HI) and rapidly digestible starch (RDS).**

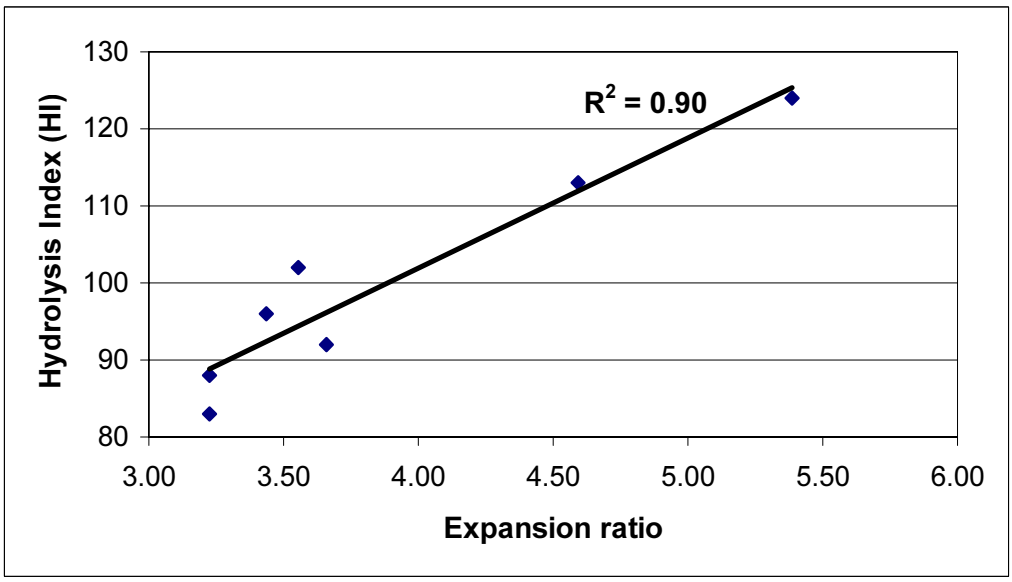


**Fig. 12. Correlation between Hydrolysis Index (HI) and expansion ratio of extrudates.**

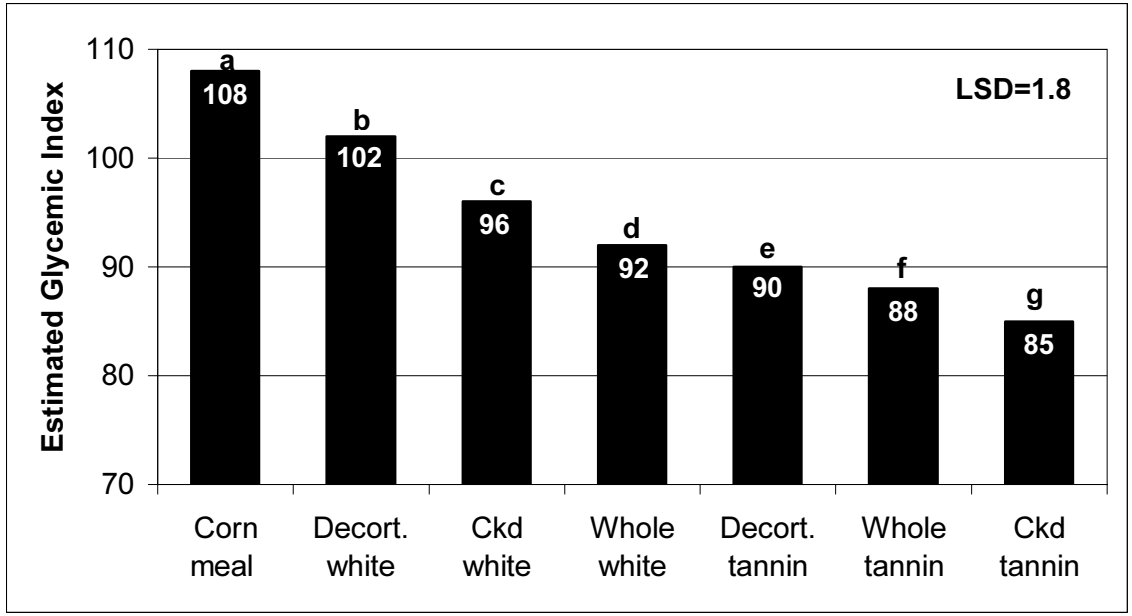


**Fig. 13. Estimated glycemic index of extrudates in descending order. Treatments with the same letter are not significantly different ($p<0.05$).**

**Table 10. Glycemic Index (GI) of some extruded or puffed products reported in the literature (from in vivo studies).**

| Product | GI* |
|---|---|
| Rice Bubbles (puffed rice, Kellogg's Inc.) | 124±6 |
| Rice Krispies (puffed rice, Kellogg's Inc.) | 117±5 |
| Coco Pops (cocoa flavored puffed rice) | 110 |
| Corn Thins (puffed corn cakes, Real Foods Co.) | 124 |
| Puffed wheat | 105±9 |
| Twisties (cheese-flavored, extruded snack, rice and corn; Smith's Snackfood Co.) | 106 |
| Cheerios (oat; General Mills Inc.) | 106±9 |
| All bran (high fiber extruded wheat-bran cereal, Kellogg's Inc.) | 60±7 |
| Ultra-bran, Vogel's, soy and linseed extruded wheat bran cereal (30.2 g fiber/100 g) (Specialty Cereals Co.) | 59 |

From Foster-Powell et al. 2002.
* Reference food: white bread (GI=100).
Note: When glucose is used as a reference food (GI of glucose=100, GI of bread=70), the GI value of the food is divided by 0.7 to obtain the GI value with white bread as a reference food.

# CHAPTER IV
# EFFECTS OF SORGHUM TYPE AND FRACTION ON STARCH AVAILABILITY OF PORRIDGES

## Introduction

Porridges prepared from wheat and rolled or flaked oatmeal are popular in many European countries and in the United States, whereas porridges prepared from sorghum, pearl millet, rice and corn are popular in many countries from Africa and Asia (Bello et al. 1990). Porridges can be thick or thin, depending on the concentration of flour. Thick porridges are “solid” and can be eaten with the fingers, while thin porridges are consumed as a beverage or with an utensil (Rooney and Waniska 2000). Traditional African thick porridges are generally made by cooking a slurry of fermented or unfermented flour in boiling water (acidic, neutral or alkaline) with continuous stirring. The porridge is cooled and eaten with a sauce (Rooney et al. 1986). Depending on the geographical region, thick porridges are called by different names such as *tộ*, *ugali*, *tuwo*, *mudde*, etc.

In some semiarid regions of Africa, sorghum thick porridges are a major portion of the total caloric intake. Sorghum cultivars that produce firm, non-sticky porridges are desired because soft and sticky porridges cannot be molded between the fingers and stick to the teeth during consumption (Bello et al. 1990). In general, white or yellow porridges are preferred, but sorghums of all colors are used to prepare porridges including tannin sorghums (Awika and Rooney 2004).

## Objective

The objective of this study was to determine the effects of sorghum type and fraction on the digestibility rates and estimated glycemic index of sorghum porridges. Corn flour was used as a reference material.

## Materials and methods

### *Grain preparation*

The sorghum types used were the white food-type (ATx635*RTx436, College Station, 2003) and the tannin “Sumac” (West Texas, 2003). A commercial refined corn meal (Cargill Inc, Minneapolis, MN) was used to prepare the corn flour. Clean whole sorghum kernels and decorticated kernels were pin milled into whole grain and decorticated (refined) flours respectively. The commercial refined corn meal was milled into corn flour using an attrition mill of 1.5 HP (Glen Mills Inc, Marywood, NJ).

### *Particle size distribution*

Particle size distribution of the sorghum and corn flours was calculated using #40, 60, 80 and 100 US standard sieves and 50 g sample size. Results were reported as percentage retained above each sieve. Measurements for each sample were made in duplicate.

### *Porridge making*

Whole and decorticated sorghum flours, and corn flour were used to prepare thick porridges. For each sample, 59 g of flour was mixed with 100 g of water to make a slurry, which was later added to 225 g of boiling water. The mixture was cooked with gentle constant stirring for 7 minutes. The samples were left to cool at room temperature for one hour. Fresh samples were used to measure starch digestibility. Subsamples were freeze dried and ground in a UDY cyclone mill (Model 3010-030, Udy Corporation, Fort Collins, CO) using a 1.0 mm round-hole screen for starch analysis.

### *Moisture*

Moisture content of the fresh porridges was determined by the two-stage air oven method (AACC Method 44-15A). Moisture content of the freeze dried samples was determined by a one-stage air oven method (AACC Method 44-19). Measurements for each sample were made in duplicate.

***Textural quality***

Textural quality (softness) of fresh porridges (cooled for 1 hr at room temperature) was evaluated using the penetrometer (Precision Penetrometer, GCA Corporation, Chicago, IL) with a 1 $^{3}/_{8}$ in. diameter cone. Based on the penetrometer readings (quadruplicate), porridge quality was evaluated according to Bello et al. (1990).

***Microscopy***

Selected samples were analyzed in a Bright Field Microscope (Zeiss Universal) using polarized light in to look at the degree of starch gelatinization in the products.

***Starch fractions***

Total starch, resistant starch and digestible starch were determined using the same methodologies described in Chapter III.

***In vitro rate of starch digestion***

The in vitro starch hydrolysis was measured in the fresh porridges (as ready to eat) as described in Chapter III using 50 mg dry weight samples.

Rapidly digestible starch (RDS), slowly digestible starch (SDS), hydrolysis index (HI) and estimated glycemic index (EGI) were obtained as described in Chapter III.

***Statistical analysis***

Mean values of all data were analyzed with one way analysis of variance (ANOVA) and significant differences were tested by Duncan's test using a confidence level of 95% ($\alpha$=0.05). The statistical software SPSS v 11.5 (SPSS Inc., Chicago, IL) was used.

**Results and discussion**

***Grain preparation and particle size distribution***

Flours with comparable particle size were obtained by milling the whole and decorticated sorghum kernels, and the corn meal (Fig. 14, Table 11). For all flours, the greatest percentage of particles was between 425 μm and 180 μm

(between US sieves #60 and #80). The corn flour had the greater percentage of particles above 425 μm (US sieve #40), while the decorticated tannin sorghum flour had the largest proportion of small particles (≤ 150 μm).

***Porridges***

Products with the characteristic appearance of thick porridges were obtained (Fig. 15). The decorticated white sorghum flour gave a white porridge, lighter than the porridge made with the whole flour due to the efficient removal of the pericarp fraction during decortication (Chapter III, Table 4). The whole tannin sorghum porridge had a dark brownish appearance due to the pigments naturally present in the pericarp and testa of this type of grain. The amount of pericarp and testa remaining after decortication of the tannin sorghum affected the final appearance of this porridge.

***Textural quality***

All sorghum porridges had significantly different softness indicated by the penetrometer readings (Fig. 16). Tannin sorghum porridges were softer (higher penetrometer readings), while corn and white sorghum porridges were firmer. Generally, soft and sticky porridges are not desired because they stick to the fingers, teeth and mouth during consumption (Rooney and Waniska 2000). Tannin sorghum porridges had a soft endosperm texture, a greater proportion of fine particles (≤150 μm), and more pericarp present in the decorticated fraction, which affected the textural quality of these products.

Starch content was negatively correlated with porridge softness ($R^2$=0.77) (Fig. 17). Bello et al. (1990) found a similar correlation when evaluating the quality of tộ (a thick porridge). According to the authors, the firmness of the porridge is related to endosperm texture, and because endosperm starch constitutes the majority of the kernel, the amount and type of starch influences the porridge quality to a large extent.

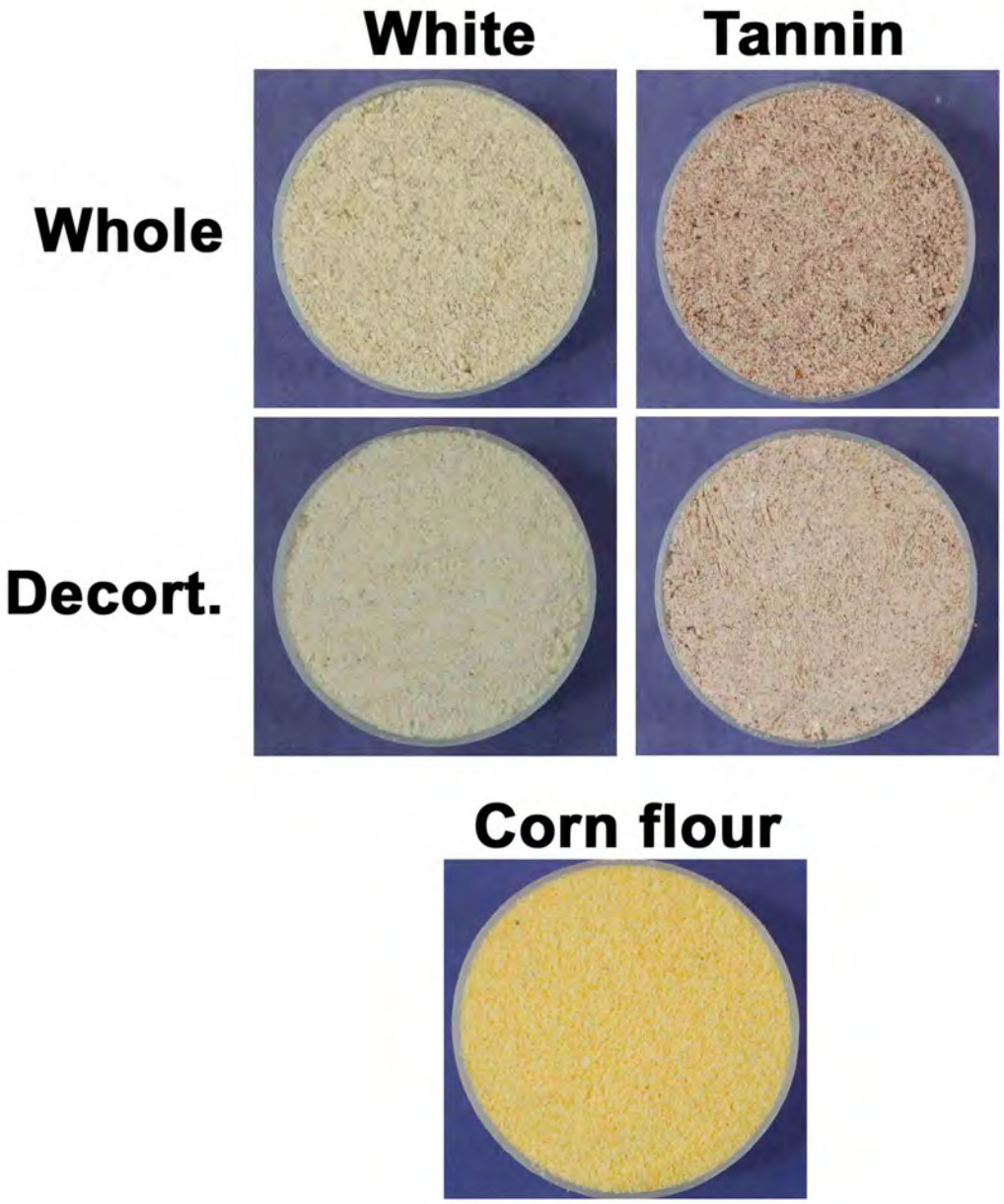


**Fig. 14. Raw materials used to prepare porridges.**

**Table 11. Particle size distribution of sorghum and corn flours used in the porridges. Treatments with the same letter within each column are not significantly different (p<0.05).**

| Sample / US Std sieve | #40 (425 µm) | #60 (250 µm) | #80 (180 µm) | #100 (150 µm) | Plate (<150 µm) |
|---|---|---|---|---|---|
| Whole white sorghum | 11.8 b | 62.1 b,c | 22.1 a | 2.6 b | 1.5 b |
| Decort. white sorghum | 7.8 c | 78.7 a | 10.8 a | 1.6 b | 1.1 b |
| Whole tannin sorghum | 4.2 d | 70.6 a,b | 18.0 a | 5.8 b | 1.5 b |
| Decort. tannin sorghum | 2.8 d | 46.2 d | 23.4 a | 18.4 a | 9.1 a |
| Corn flour | 21.0 a | 51.1c,d | 24.7 a | 2.4 b | 1.2 b |
| LSD | 1.9 | 14.8 | 13.3 | 5.5 | 5.8 |

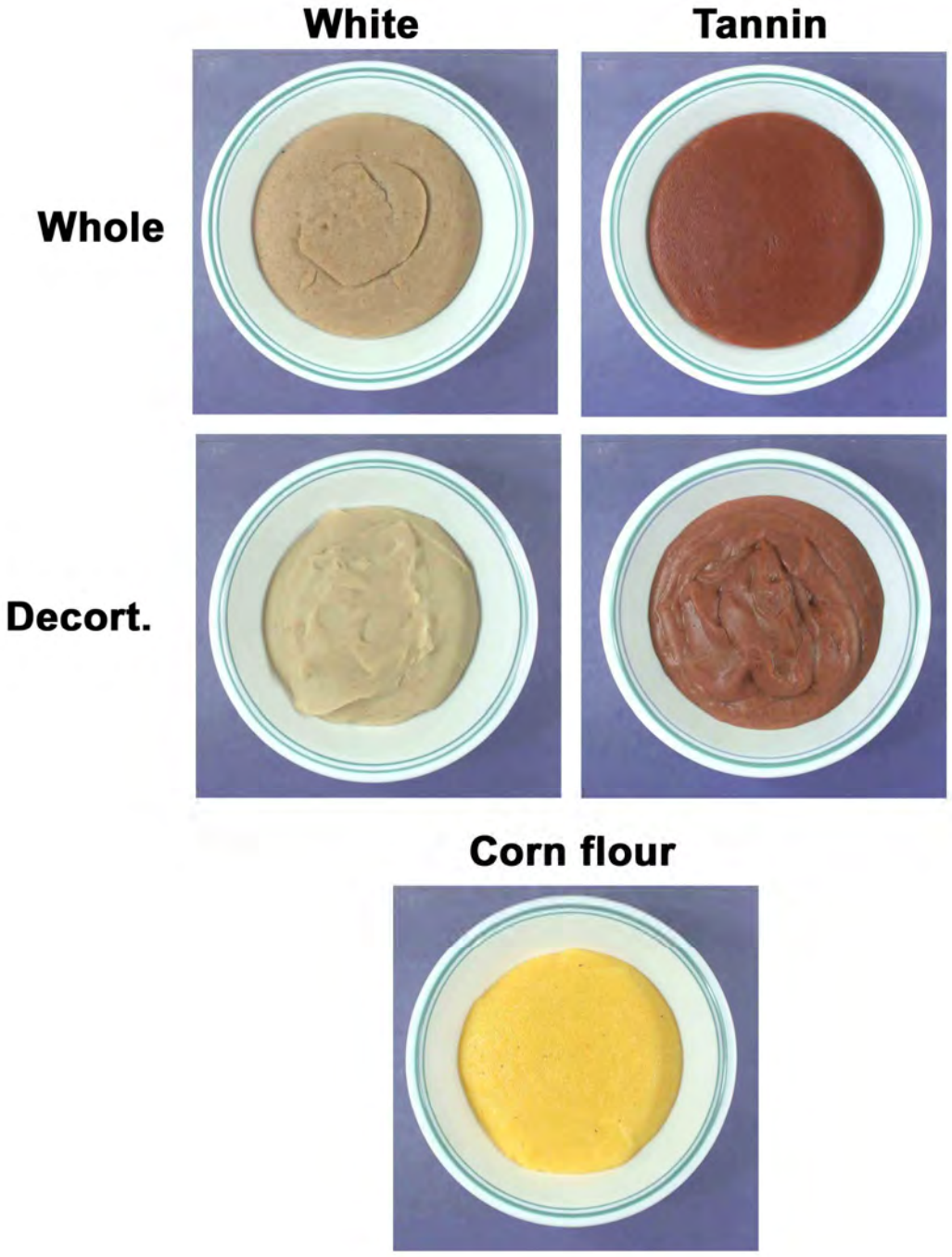


**Fig. 15. Final appearance of the fresh porridges.**

Stickiness of cooked sorghum flour is a function of starch gelatinization (Hoseney 1986). When made into a porridge, hard grains form products that are less sticky than grains with a larger proportion of floury endosperm; protein may influence this behavior by interfering with starch gelatinization (Cagampang and Kirelis 1985).

Corn flour formed the hardest porridge (Fig. 16). Bello et al. (1990) observed that the presence of more bran (pericarp) in flours yield softer gels when preparing thick porridges. In the decorticated sorghum fractions not all the bran was removed (e.g. they are partially refined flours), while the commercial corn flour used in the corn porridge was highly refined, and no bran pieces affected the texture of the porridge.

***Microscopy***

Porridges were analyzed using light microscopy (Fig. 18). The whole and decorticated white sorghum porridges show intact starch granules that are fully gelatinized (Fig. 18, B and D). The tannin sorghum porridges show fully gelatinized starch granules that are more disrupted (Fig. 18, A). The higher proportion of corneous to floury endosperm in the white sorghum affected the starch gelatinization as suggested by Cagampang and Kirelis (1985). The corneous endosperm contains abundant protein bodies that surround the starch granule, whereas the floury endosperm is relatively free of protein bodies. Thus, the protein in the white sorghum (hard endosperm) influenced starch gelatinization. Bran pieces are in both types of sorghum porridges. Corn flour porridge shows starch granules that are gelatinized and partially dispersed in a matrix. However, unlike sorghum porridges, corn porridge has greater proportion of retrograded starch while bran pieces are not noticeable (Fig. 18, C).

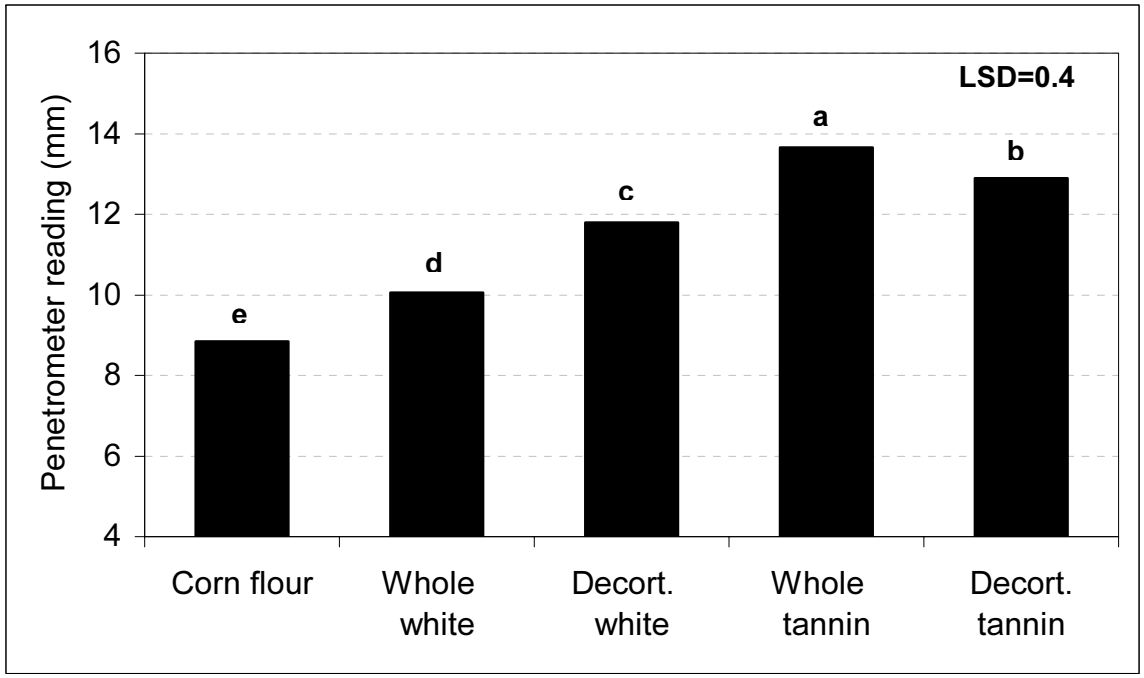


**Fig. 16. Texture quality (softness) of fresh porridges based on penetrometer readings.**

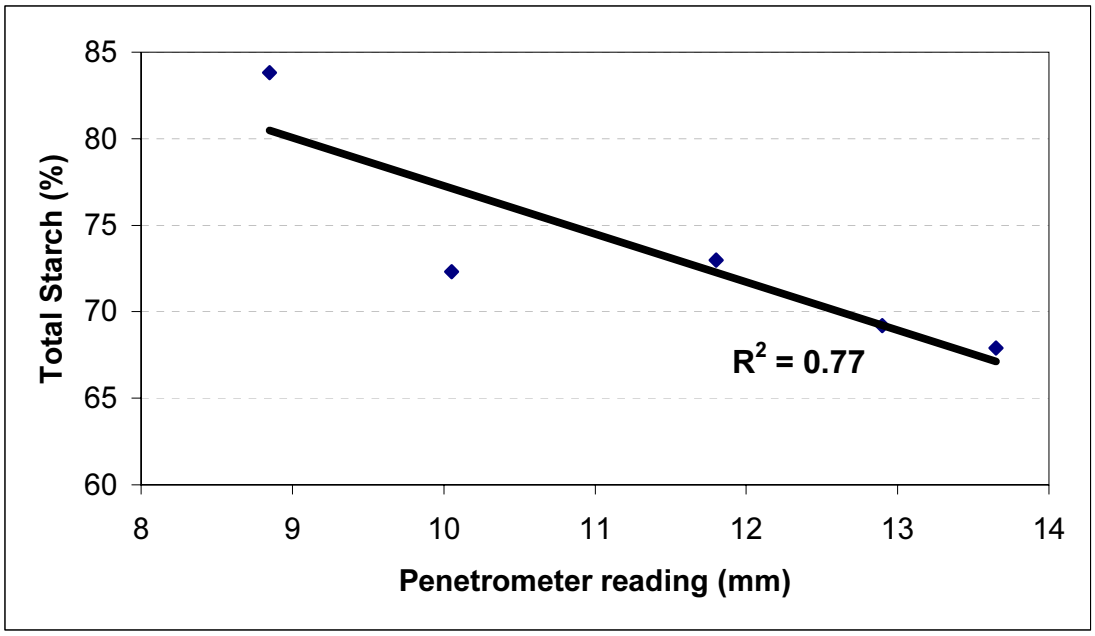


**Fig. 17. Correlation between starch content and softness of fresh porridges.**

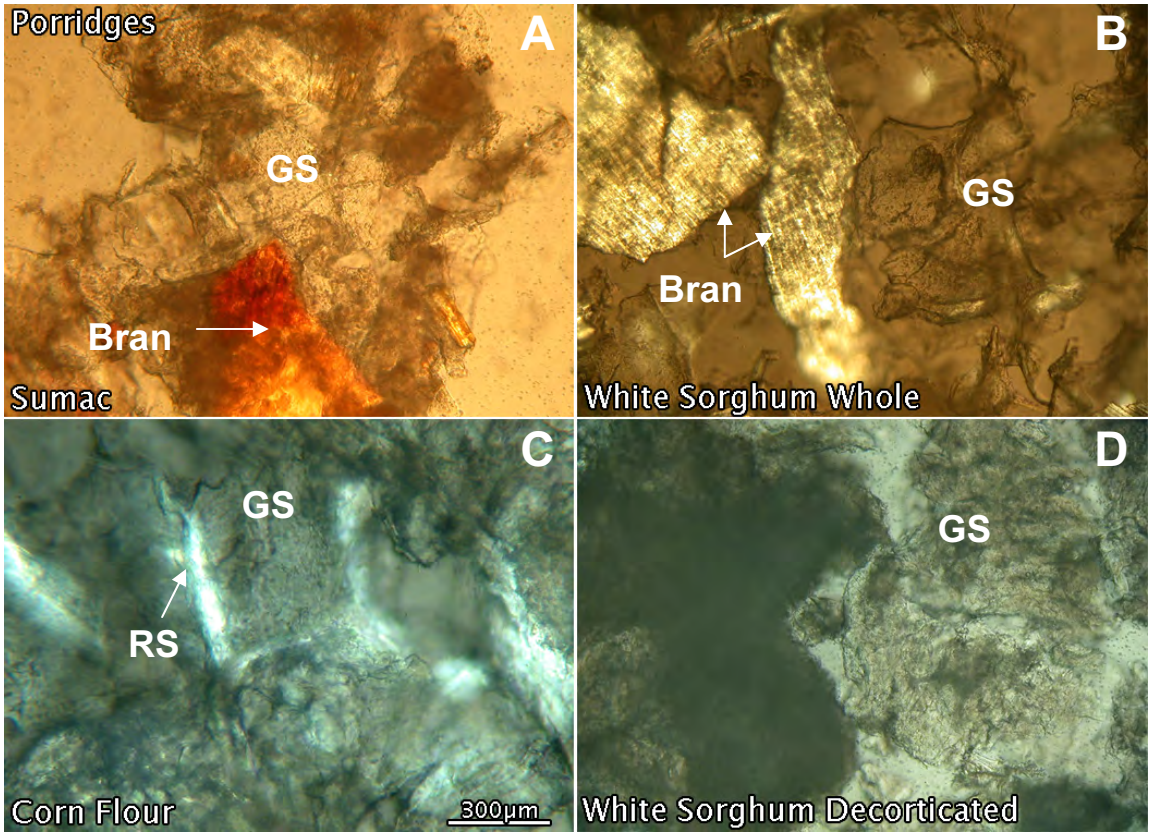


**Fig. 18. Selected porridges seen under light microscopy. A: Whole tannin sorghum, B: Whole white sorghum, C: Corn flour, D: Decorticated white sorghum. RS: Retrograded starch, GS: gelatinized starch.**

***Starch fractions***

Total starch (TS) values ranged from 67.9% to 83.8% on dry matter basis. The more refined the grain (e.g. corn flour) the higher the amount of TS in the products (Table 12). White sorghum was decorticated at 9%, without significantly affecting the amount of TS of the flour. Tannin sorghum lost 18% during decortication, significantly concentrating the TS fraction in the porridge from decorticated flour.

The resistant starch (RS) values were significantly different for most of the samples. The corn porridge had the highest amount of RS (4.0%). Rosin et al. (2000) reported that polenta made with corn meal (similar to a thick porridge) has 4.2% of RS. On the other hand, sorghum porridges had comparable RS contents (between 2.2% and 2.9%) except for the whole white sorghum porridge which had a significantly lower RS content. The starch fraction considered digestible (DS) was high for all the samples

***In vitro rate of starch digestion***

Fig. 19 shows the starch digestion curves of fresh porridges (cooled for 1 h). The corn flour porridge had a higher starch digestibility compared to all the sorghum porridges (Table 13). According to Zhang and Hamaker (1998), pure maize starch is more digestible than pure sorghum starch. Among the sorghum porridges, lower digestibilities were observed when using the whole flours compared to decorticated flours for each type of sorghum. A higher content of dietary fiber and bigger particles (>450 μm) of the whole flours are factors involved in the reduced digestibility observed in the whole porridges. Previous studies have confirmed that using whole grains and coarse-grain pieces in food products reduced the starch digestibility compared to using the processed grains (e.g. refined/fine flours) (Witwer 2005, Foster-Powell 2002).

When comparing sorghum types, the tannin porridges had an initial higher rate of digestion compared to the white sorghum porridges for both fractions (whole and decorticated). However, after 3 h of digestion, the white

sorghum porridges had a higher total percentage of starch digested compared to the tannin porridges. Endosperm texture could explain the initial fast digestibility rate for the tannin porridge because the soft endosperm was easier to gelatinize and available for digestion. On the other hand, proteins in the hard endosperm of white sorghum created a barrier to delay the gelatinization and/or enzyme access to the starch.

Particle size could also influence the initial rate of digestion of the decorticated tannin sorghum porridge, since it had the smallest particle size among the flours (Table 11, Fig. 19). Van der Merwe et al. (2001) suggested that in a porridge, enzyme accessibility to starch has to do with the contact surface area of the enzymes with the substrate (starch). During the digestion of a porridge matrix, the enzymes contact the outer surface of the endosperm particles, and as these particles are released, the contact surface area of the enzymes with the substrate would increase. The large effect of particle size was confirmed by preparing porridges using corn meal vs. corn flour (APPENDIX A).

After 60 min of incubation, tannin sorghum porridges reach a steady state of starch digestion, while white sorghum porridges had increased digestibility with a higher percentage of starch digested (Fig 19, Table 13). Condensed tannins in the tannin sorghum could have interfered with starch digestion. Furthermore, Bach Knudsen et al. (1988) concluded that a higher content of dietary fiber, resistant starch, and kafirin proteins decrease starch digestibility of *ugali* (a thick sorghum porridge).

In this study, no correlation between higher RS and lower digestibility was found. In contrast, the whole white sorghum porridge had slower rate of starch digestion and the lowest RS, while the corn porridge had the highest rate of starch digestion and the highest RS (Fig 19, Table 12). According to van der Merwe et al. (2001), during the cooling period, porridges with the most disrupted starch granules (solubilized amylose leached out) produced more retrograded amylose (type 3 RS).

**Table 12. Moisture and starch fractions of porridges (% dry weight basis).**

| Type of porridge | % Moisture* | Total starch | Resistant starch | Digestible starch |
|---|---|---|---|---|
| Corn flour | 84.5 a | 83.8 a | 4.0 a | 79.8 a |
| Whole white sorghum | 85.5 a | 72.3 b | 0.5 d | 71.8 b |
| Decort. white sorghum | 85.0 a | 73.0 b | 2.9 b | 70.1 c |
| Whole tannin | 85.0 a | 67.9 d | 2.5 c | 65.4 e |
| Decort. tannin | 84.2 a | 69.2 c | 2.2 c | 67.0 d |
| LSD | 1.3 | 0.9 | 0.3 | 1.1 |

*: Moisture of fresh porridges.

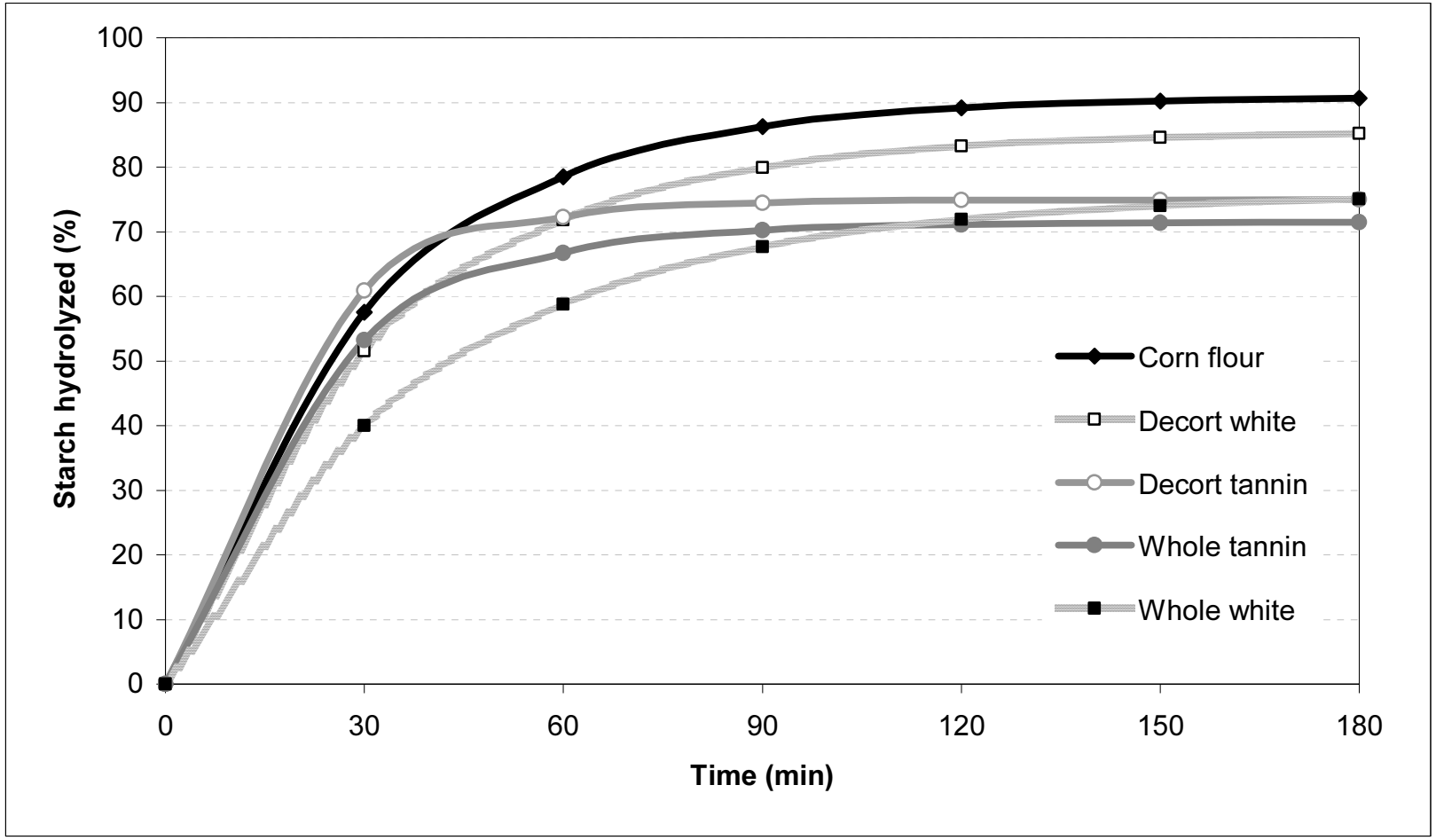


**Fig. 19. In vitro starch digestibility of porridges from 0 to 180 minutes.**

**Table 13. Percentage (dry wt basis) of starch hydrolyzed at different times (min), and calculated C∞ and k constants for each type of porridge. Treatments with the same letter within each column are not significantly different (p<0.05).**

| Type of porridge | Time (min) 30 | 60 | 90 | 120 | 150 | 180 | C∞ | k |
|---|---|---|---|---|---|---|---|---|
| Corn flour | 57.3 b | 78.6 a | 86.6 a | 89.6 a | 90.7 a | 91.2 a | 91.5 a | 0.032 c |
| Whole white | 40.0 d | 58.7 d | 67.6 e | 71.9 d | 74.0 c,d | 75.0 c | 76.1 c | 0.025 d |
| Decort. white | 51.5 c | 71.8 b | 79.9 b | 83.2 b | 84.6 b | 85.2 b | 85.6 b | 0.033 c |
| Whole tannin | 53.2 c | 66.7 c | 70.2 d | 71.1 d | 71.3 d | 71.4 c | 71.4 d | 0.045 b |
| Decort. tannin | 60.9 a | 72.3 b | 74.4 c | 74.8 c | 74.9 c | 74.9 d | 74.9 c,d | 0.055 a |
| LSD | 3.6 | 2.7 | 2.4 | 2.8 | 3.2 | 3.5 | 3.8 | 0.006 |

The kinetic parameters that describe the hydrolytic process of starch digestion were obtained (Table 13). C∞ stands for the equilibrium concentration, reached after 180 min of hydrolysis and the constant "k" stands for the kinetic (digestibility) constant. Corn and decorticated white sorghum porridges had similar starch digestion patterns and gave similar kinetic constants (i.e. the intrinsic susceptibility of the starch in the products to digestion was similar). This finding suggests that using the refined fractions of corn and white sorghum enhanced the starch digestibility (less amount of other flour components such as fiber, lipids, ash, minerals). However, the lower overall digestibility of the decorticated white sorghum porridge compared to the corn porridge was influenced by extrinsic factors. Cooking has shown to significantly reduce protein digestibility of sorghum but not of corn, because of formation of disulfide cross links within, and possible between, protein bodies (Zhang and Hamaker 1998). These protein complexes could interfere with starch digestibility. On the other hand, the kinetic constants of whole white, whole tannin, and decorticated tannin porridges were significantly different, suggesting that the differences in digestibility were due to the innate properties of the starches in the products.

*Rapidly and slowly digested starch*

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were calculated from the in vitro starch digestion at 30 and 120 min of enzymatic incubation respectively (Fig. 20). RDS was higher for the tannin sorghum porridges compared to the white sorghum porridges. The RDS of the sorghum porridges was correlated with the softness ($R^2$=69), suggesting that endosperm-texture properties caused the differences in the RDS fractions of both types of sorghum. (Fig. 21). According to Zhang and Hamaker (1998), protein in cooked sorghum flour pastes plays an important role in making slowly digesting starch. The tight packing of starch in the protein matrix in the hard endosperm of white sorghum could limit the distortion of starch granules (Van der Merwe et al. 2001), whereas the starch in the tannin sorghum porridge was easy to

gelatinize, because of the looser packing of the starch granules (soft endosperm).

On the other hand, the reduced SDS fraction of the tannin sorghum porridges suggests that the condensed tannins from tannin sorghum porridges could have formed complexes with protein/starch, reducing the amount of starch available for digestion (i.e. less total percentage of starch digested after 3 h compared to the white sorghum products) (Fig 20, Table 13).

*Hydrolysis index and estimated glycemic index*

The hydrolysis index (HI) was used in the formula by Goñi et al. (1997) to obtain the estimated Glycemic Index (EGI). The EGI of all products was significantly different (Table 14). While the HI of the extrudates were highly correlated with RDS (Chapter III, Fig. 11); in the case of the porridges this correlation was low ($R^2$=0.45). In contrast, the HI value of the porridges was highly correlated with the SDS fraction ($R^2$=0.87) (Fig. 22), meaning that the percentage of starch hydrolyzed after 2 h of digestion (120 min) was a better predictor of the EGI of the porridges.

Flours that were prepared and cooked into porridges, showed different estimated glycemic index (EGI) values (Table 14). This confirms that botanical differences in grains processed the same way results in differences in the degree of starch gelatinization, starch digestion, and consequently the glycemic index (GI) values (Foster-Powell et al. 2002). The factor with greatest effect on porridge digestibility and EGI values was the grain fraction used. All sorghum samples had statistically different EGI. When decorticated (removal of the bran fraction), both types of sorghum showed an increase in the EGI values.

When organizing the EGI in descendent order (Fig. 23), corn flour porridge had the highest value (101), followed by the decorticated white (98), decorticated tannin (95), whole tannin (91), and whole white (87) sorghum porridges.

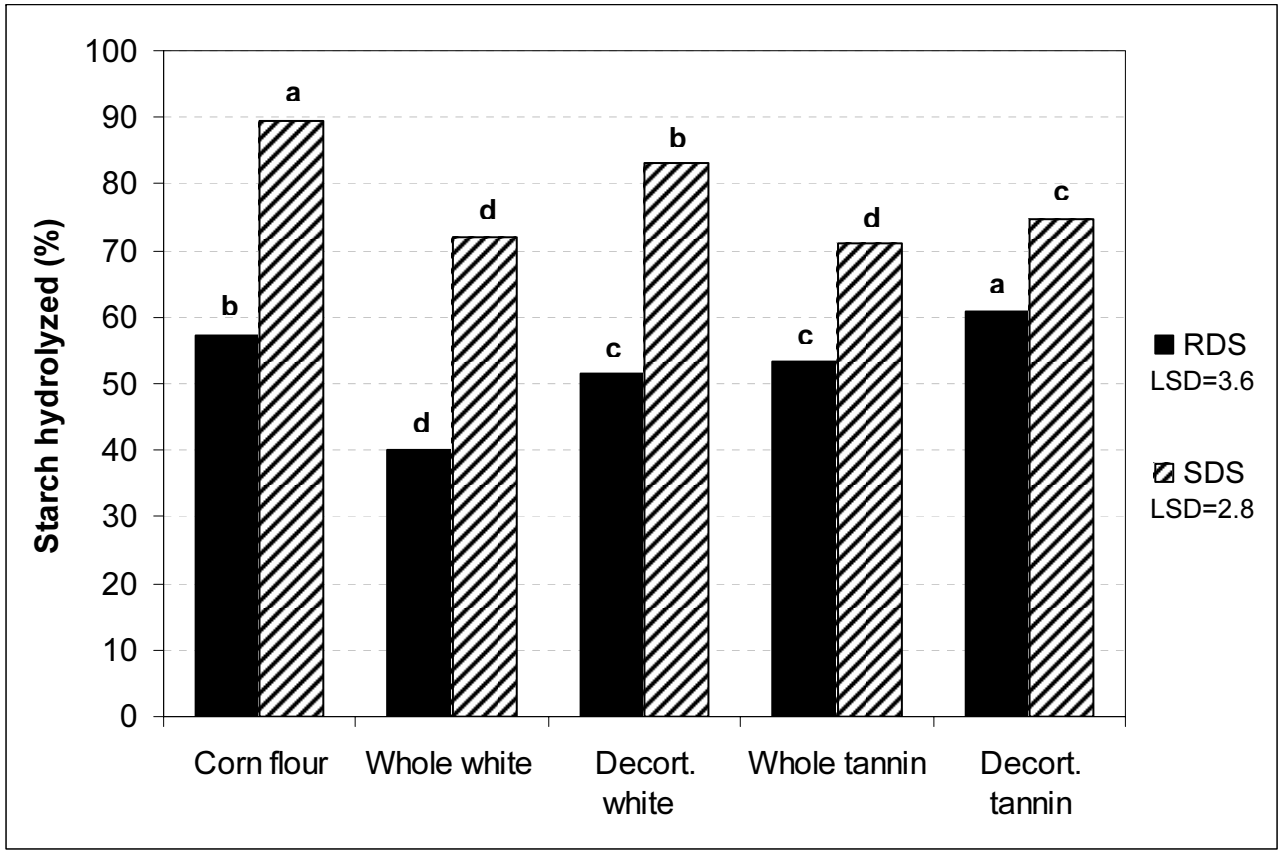


**Fig. 20. Rapidly digestible starch (RDS), and slowly digestible starch (SDS) of porridges (dry wt basis). Treatments with the same letter within each type of bar are not significantly different ($p<0.05$).**

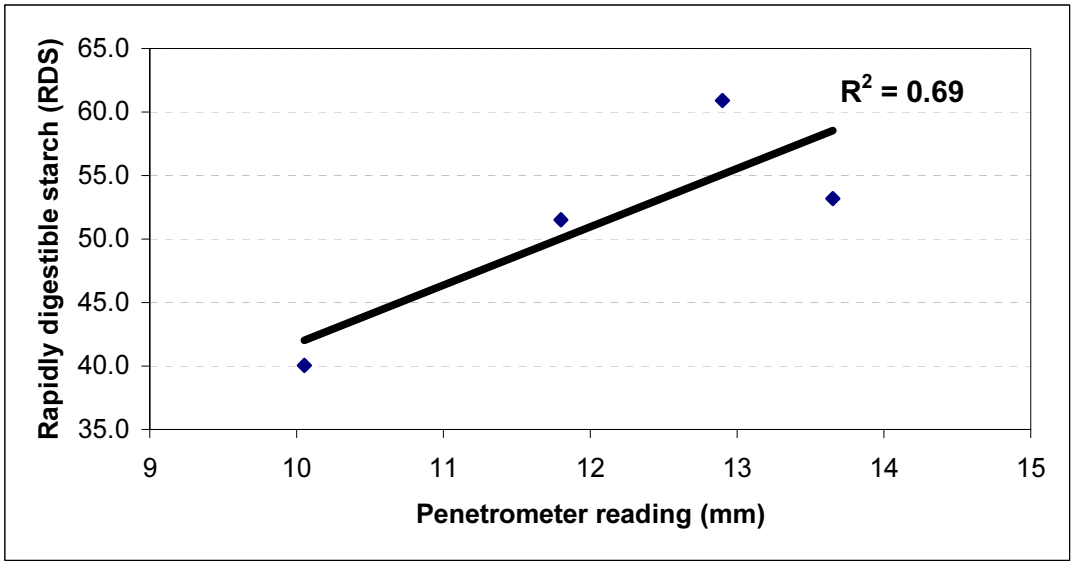


**Fig. 21. Correlation between rapidly digestible starch (dry wt. basis) and softness of sorghum porridges.**

**Table 14. Hydrolysis Index (HI) and estimated Glycemic Index (EGI) of porridges.**

| | HI* | EGI* |
|---|---|---|
| Corn flour | 112 ±1a | 101 ±1a |
| Whole white sorghum | 87 ±2 e | 87± 1 e |
| Decort. white sorghum | 106 ±2 b | 98 ±1 b |
| Whole tannin sorghum | 93 ±3 d | 91 ±2 d |
| Decort. tannin sorghum | 100 ±3 c | 95 ±2 c |
| LSD | 3.0 | 1.7 |

*: ±SD.

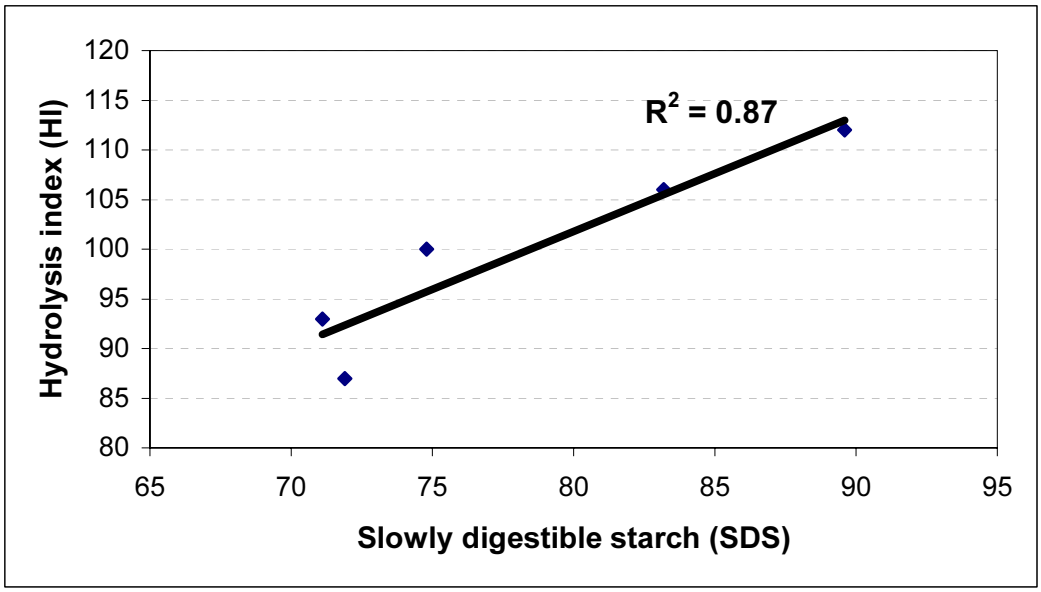


**Fig. 22. Correlation between Hydrolysis Index (HI) and slowly digestible starch (SDS).**

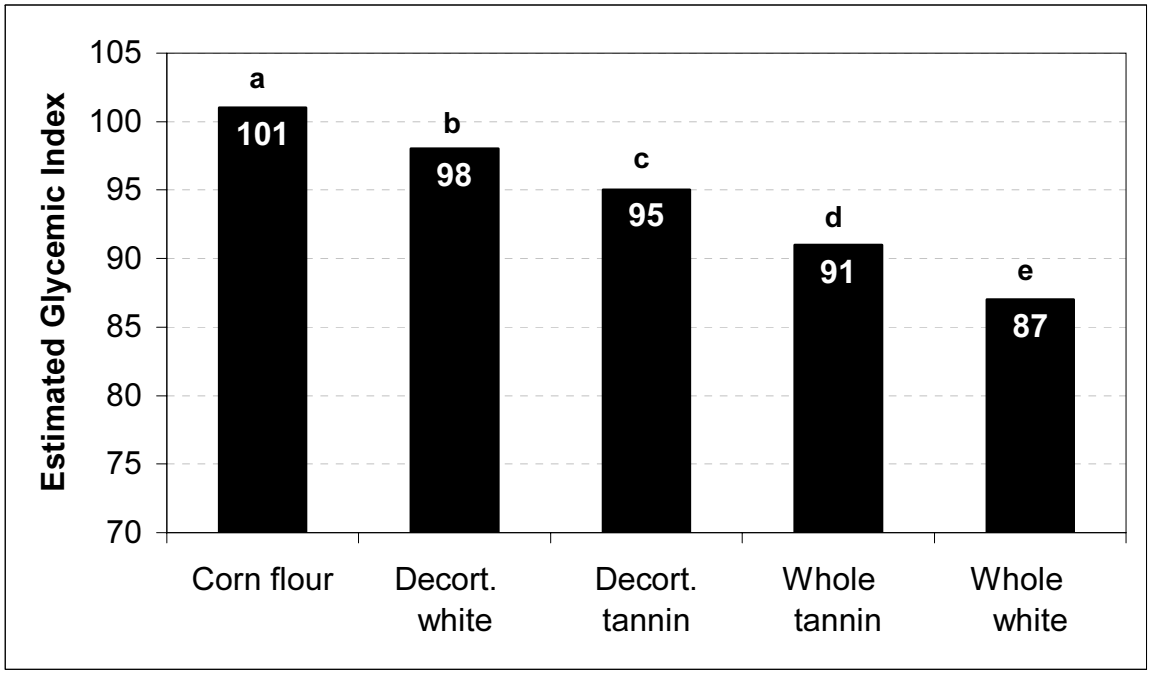


**Fig. 23. Estimated glycemic index of porridges in descending order.**

**Table 15. Glycemic Index (GI) of some cereal porridges reported in the literature (from in vivo studies).**

| Product | GI* |
|---|---|
| Millet flour porridge (Kenya) | 153±14 |
| Maize meal porridge or gruel (Kenya) | 156±15 |
| Maize meal porridge, refined maize meal:water (1:3) (South Africa) | 106 |
| Maize meal porridge, unrefined maize meal:water (1:3) (South Africa) | 101 |
| Whole-meal oat-flour porridge (flour:water, 1:3), boiled 2.5 min (Sweden) | 106±19 |
| Whole-meal barley flour porridge (100% regular barley) (flour:water, 1:3), boiled 2.5 min (Sweden) | 97±16 |
| Whole-meal high-fiber barley flour porridge (50% regular barley flour, 50% high fiber barley flour) (Sweden) | 78±8 |
| Multigrain porridge cooked with water (contains rolled oats, wheat, triticale, rye, barley and rice): The Monster Muesly Co. NSW, Australia | 79 |

From Foster-Powell et al. 2002.
* Reference food: white bread (GI=100).
Note: When glucose is used as a reference food (GI of glucose=100, GI of bread=70), the GI value of the food is divided by 0.7 to obtain the GI value with white bread as a reference food.

The EGI of the sorghum porridges can be compared to the GI of porridges reported in the literature (Table 15). Decorticated sorghum porridges had lower EGI values compared to the GI of porridges from refined flours, such as corn and millet. Whole sorghum porridges had comparable EGI values to whole-oat flour and whole-barley flour porridges.

# CHAPTER V
# EFFECT OF TANNIN SORGHUM BRAN ADDITION ON STARCH AVAILABILITY OF WHEAT BREAD

## Introduction

Over the past decade, the production of nonwhite or specialty breads has rapidly increased (Hoseney 1994). Whole-grain and high-fiber breads have gained popularity among consumers due to the existing awareness of the health benefits of consumption of dietary fiber. Sources of fiber have been added to breads successfully (Park et al. 1997). Substituting part of the wheat flour with fiber ingredients weakens the gluten matrix reducing loaf volume; therefore, vital wheat gluten is frequently used to improve the quality of these type of breads (Czuchajowska and Pomeranz 1993, Hoseney 1994).

Condensed tannins are the major phenolic compound of sorghums with pigmented testa. Awika (2003) found that when the grain is decorticated, the condensed tannins are concentrated in the bran fraction. Sorghum bran fractions (pericarp and testa) contain 36-50% dietary fiber (Rooney et al. 2002), and an antioxidant potential that exceeds high-antioxidant fruits such as blueberries and strawberries on a dry weight basis (Awika 2003). High tannin sorghum bran has been successfully incorporated into good quality breads (Gordon 2001) and bread mixes (Rudiger 2003), as a natural source of brown color, antioxidants and dietary fiber.

## Objective

The objective of this study was to determine the effect of tannin sorghum bran addition on the starch digestibility and estimated glycemic index of wheat bread.

## Materials and methods

### *Sorghum grain preparation*

Tannin sorghum (Sumac, West Texas, 2003) was decorticated in 4-kg batches in a PRL mini-dehuller (Nutama Machine Co., Saskatoon, Canada) to remove the bran fraction. The bran was removed with a KICE grain cleaner (Model 6DT4-1, KICE Industries Inc., Wichita KS). The bran obtained (~18% original weight) was further milled into a fine powder using the Pin Mill.

### *Tannin sorghum bran characterization*

Particle size distribution of the bran was calculated using #40, 60, 80, and 100 US standard sieves and 50 g sample size. Dietary fiber was determined following the standard analytical procedure (AACC Method 32-05). Bran color was determined with a colorimeter (model CR-310, Minolta C0., LTD. Ramsey, NJ), using the CIE-L* a* b* uniform color space (CIE-Lab). Awika's (2003) modification of the Folin-Ciocalteu method of Kaluza et al. (1980) was used to determine phenols. Extraction was performed using 1% HCl in methanol as solvent. Bran was analyzed for antioxidant activity with the ABTS [2,2´- azinobis (3-ethyl-benzothiaziline-6-sulfonic acid)] method as described by Awika et al. (2003).

### *Bread baking*

The wheat bread formulation consisted of refined wheat flour (Gold Medal Superlative, General Mills Inc, Minneapolis, MN), sugar, salt, yeast, SSL, oil and water (Table 16). The ingredients were added using baker's percentages. For the treatment formulation, 12% of the wheat flour was substituted with tannin sorghum bran plus 1% of vital wheat gluten. The dry ingredients were weighed, mixed together, and placed in a bread machine TR800 Breadman Plus automatic bread maker (Salton/MAXIM Housewares Inc., Mt. Prospect, IL); water and oil was added into the bread pan. The bread baking was done by selecting the normal cycle (medium crust). After the cycle was complete (3:10 h), the bread loaf was placed in a cooling rack until completely cool. Fresh

samples from the crumb of the bread were used to measure starch digestibility. Subsamples were freeze dried and ground in a UDY cyclone mill (Model 3010-030, Udy Corporation, Fort Collins, CO) using a 1.0 mm round-hole screen for the analysis of the starch fractions.

***Moisture***

Moisture content of the fresh prepared breads was determined by the two-stage air oven method (AACC Method 44-15A). Moisture content of the freeze dried samples was determined by the one-stage air oven method (AACC Method 44-19). Measurements for each sample was made in duplicate.

***Starch fractions***

Total starch, resistant starch and digestible starch were determined using the same methodologies described in Chapter III.

***In vitro rate of starch digestion***

The in vitro starch hydrolysis was measured as described on Chapter III, using 50 mg dry weight sample from the crumb of the fresh prepared breads. Rapidly digestible starch (RDS), slowly digestible starch (SDS), hydrolysis index (HI) and estimated glycemic index (EGI) were obtained as described in Chapter III.

***Statistical analysis***

Mean values of all data were analyzed with one way analysis of variance (ANOVA) and significant differences were tested by Duncan's test using a confidence level of 95% ($\alpha$=0.05). The statistical software SPSS v 11.5 (SPSS Inc., Chicago, IL) was used.

**Table 16. Bread formula.**

| Ingredients | Flour % |
|---|---|
| Refined wheat flour | 100 |
| Sugar | 6.0 |
| Salt | 1.5 |
| Active dry yeast | 0.8 |
| Sodium stearoyl lactylate (SSL) | 0.3 |
| Oil | 3.0 |
| Water | 62 |
| Tannin sorghum bran [a] | 12 |
| Vital wheat gluten [b] | 1.0 |

[a] Substituted as part of the wheat flour.
[b] When tannin bran was added.

## Results and discussion

### *Tannin sorghum bran characterization*

Tannin sorghum bran characteristics are shown in Table 17. The tannin bran was a fine brown powder (90% passed trough a US sieve #100). The phenols and antioxidant activity values were high due to the concentration of the condensed tannins in the decortication process.

### *Breads*

A wheat bread and a bread containing 12% sorghum bran were obtained (Fig. 24). For the wheat bread, a refined wheat flour was used, giving the conventional white bread appearance. On the other hand, the bread with 12% tannin sorghum bran had a natural dark brown color due to the pigments present in the bran fraction (testa and pericarp).

### *Starch fractions*

Total starch (TS), resistant starch (RS) and digestible starch (DS) fractions of the two types of breads are shown in Table 18. The bread with added tannin bran had a significantly lower amount of TS due to the substitution of the bran fraction for the refined wheat flour. The RS of the wheat bread was similar to previous studies. Rosin et al. (2002) reported that white bread had a RS of 0.8%, while Brown (2004) reported 0.9%, all on dry weight basis. Adding the tannin bran to the bread slightly increased the amount of RS; however, this increase was not statistically different.

### *In vitro rate of starch digestion*

The bread with tannin bran had a slower rate of starch digestion compared to the regular wheat bread (Fig. 25). Except for the initial 30 min of hydrolysis, at all sampling times, lower percentages of starch were hydrolyzed in the product with tannin bran compared to the wheat bread sample (Table 19).

Goñi et al. (1996) found 76.1% starch digestion for wheat bread after 180 min. In this study, wheat bread had a similar starch digestion (78.4%), although different formulations were used. On the other hand, tannin-bran bread had a

significantly lower percentage of starch digested. The tannin bran was rich in condensed tannins, which could interfere with wheat starch digestion (Chung et al. 1998). Increased amounts of dietary fiber and larger particle size of the tannin bran fraction could also hinder starch digestion. Furthermore, the tannin bran was also a source of sorghum starch, which could be more slowly digested than wheat starch.

Bread is an open structure with many air holes. According to van der Merwe et al. (2001), the porous structure of bread increases the contact surface area of the sample with the enzymes during digestion. A great accessibility of starch in the bread contributes to the high rate of starch digestibility during the digestion period.

The kinetic parameters that describe the hydrolytic process of starch digestion were obtained (Table 19). The white bread and the tannin-bran bread gave identical kinetic constants. This suggests that the addition of tannin bran did not alter the intrinsic susceptibility of the wheat starch to digestion. Therefore, it confirmed that differences in the digestibility of the two breads were due to extrinsic factors (i.e. condensed tannins, dietary fiber, starch of tannin bran).

*Rapidly and slowly digested starch*

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were calculated from the in vitro starch digestion at 30 and 120 min of enzymatic incubation respectively (Fig. 26). The initial rate of digestion and the RDS was the same for both types of breads. After 30 min, the rate of digestion of the tannin-bran bread was lower for all sampling times compared to the wheat bread. Consequently the SDS was significantly different.

*Hydrolysis index and estimated glycemic index*

The estimated Glycemic Index (EGI) of each bread was obtained by using the hydrolysis index (HI) value in the formula by Goñi et al. (1997). The bread with the added tannin bran had a significantly lower EGI compared to the wheat

**Table 17. Tannin sorghum bran characterization.**

| | **Tannin bran** |
|---|---|
| Dietary fiber (% d.b) | 38.2 |
| Phenols (mg GAE/g) | 90.9 |
| Antioxidant Activity (μmol TE/g) | 1190.7 |
| Color | |
| L* | 50.5 |
| a* | 10.2 |
| b* | 11.7 |
| Particle size distribution (%weight) | |
| US sieve #40 (425 μm) | 0.4 |
| US sieve #60 (250 μm) | 1.4 |
| US sieve #80 (180 μm) | 4.7 |
| US sieve #100 (150 μm) | 2.9 |
| Pan (<150 μm) | 90.6 |

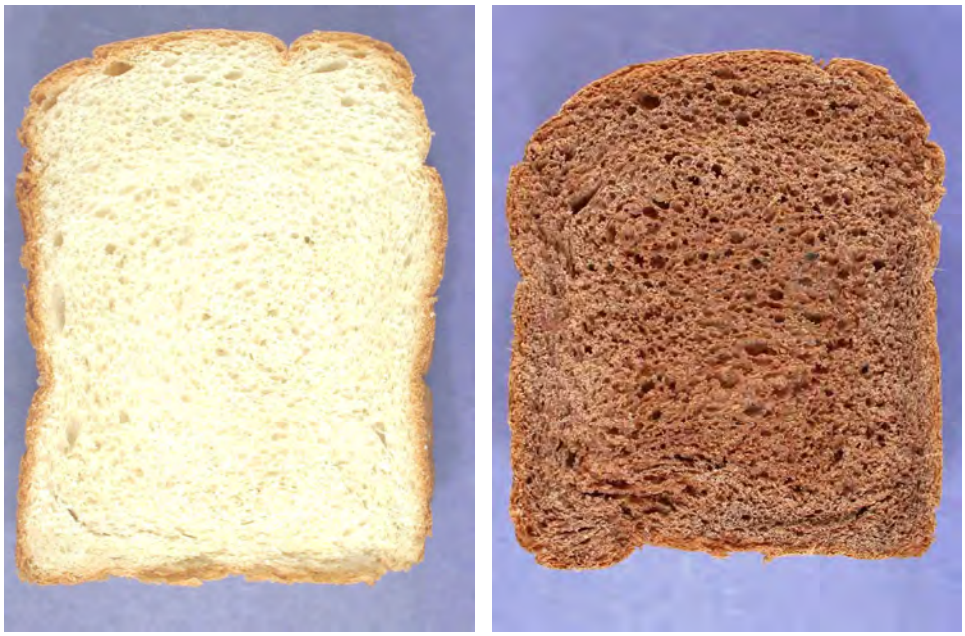

**Fig. 24. Physical appearance of the white bread (left) and bread with 12% added tannin sorghum bran (right).**

**Table 18. Moisture and starch fractions of breads (% dry weight basis).**

| Type of bread | % Moisture | Total starch | Resistant starch | Digestible starch |
|---|---|---|---|---|
| White bread | 44.7 a | 69.8 a | 1.0 a | 68.8 a |
| Bread 12% tannin sorghum bran | 44.3 a | 63.0 b | 1.2 a | 61.8 b |
| LSD | 1.3 | 1.0 | 0.3 | 1.0 |

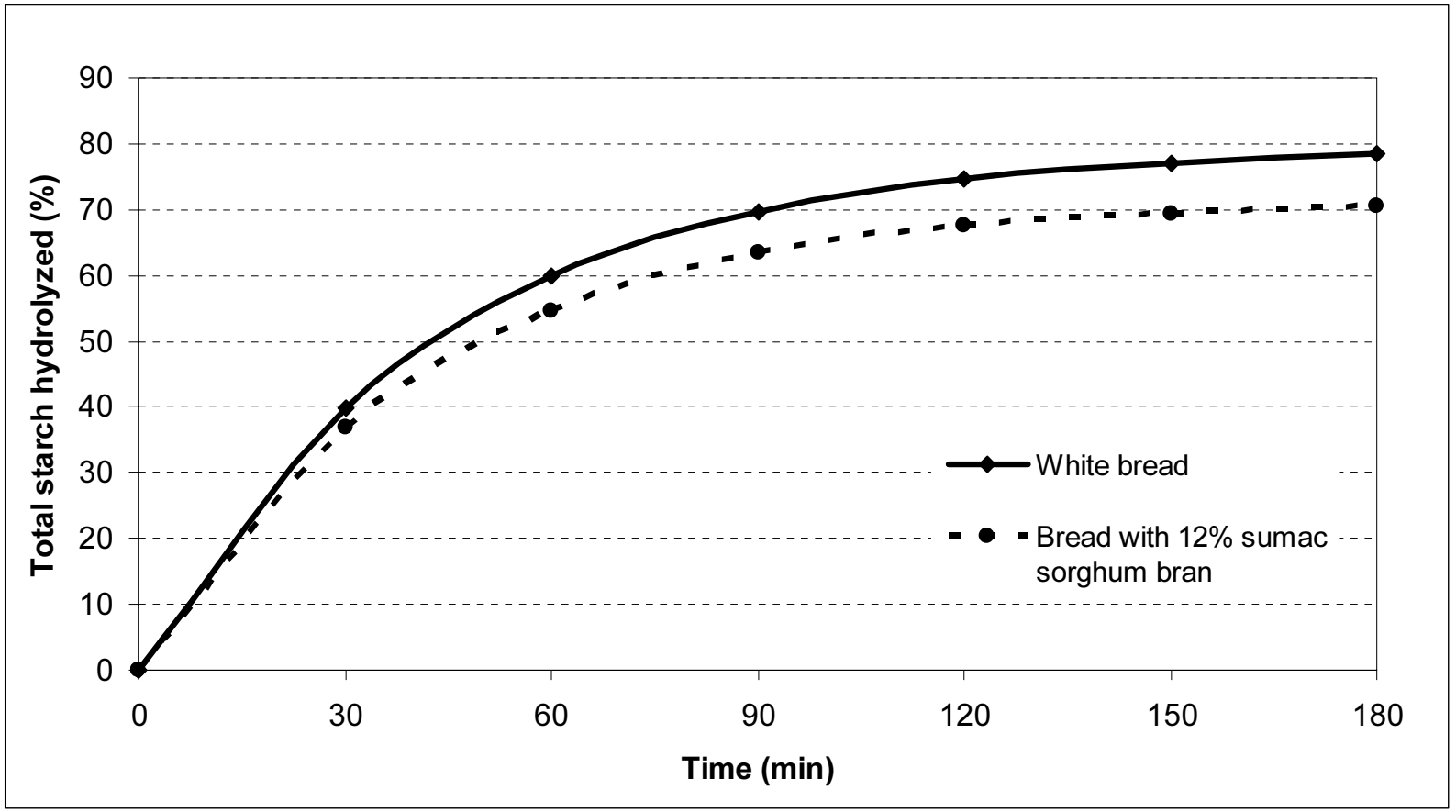


**Fig. 25. In vitro starch digestibility of breads from 0 to 180 minutes.**

**Table 19. Percentage (dry wt basis) of total starch hydrolyzed at different times (min), and calculated C∞ and k constants for each type of bread.**

| Type of bread | Time (min) | | | | | | C∞ | k |
|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | | |
| White bread | 39.9 a | 59.8 a | 69.7 a | 74.6 a | 77.1 a | 78.4 a | 79.6 a | 0.023 a |
| Bread 12% tannin sorghum bran | 36.9 a | 54.7 b | 63.3 b | 67.5 b | 69.5 b | 70.5 b | 71.4 b | 0.025 a |
| LSD | 2.2 | 2.4 | 2.3 | 2.5 | 2.6 | 2.8 | 3.1 | 0.0 |

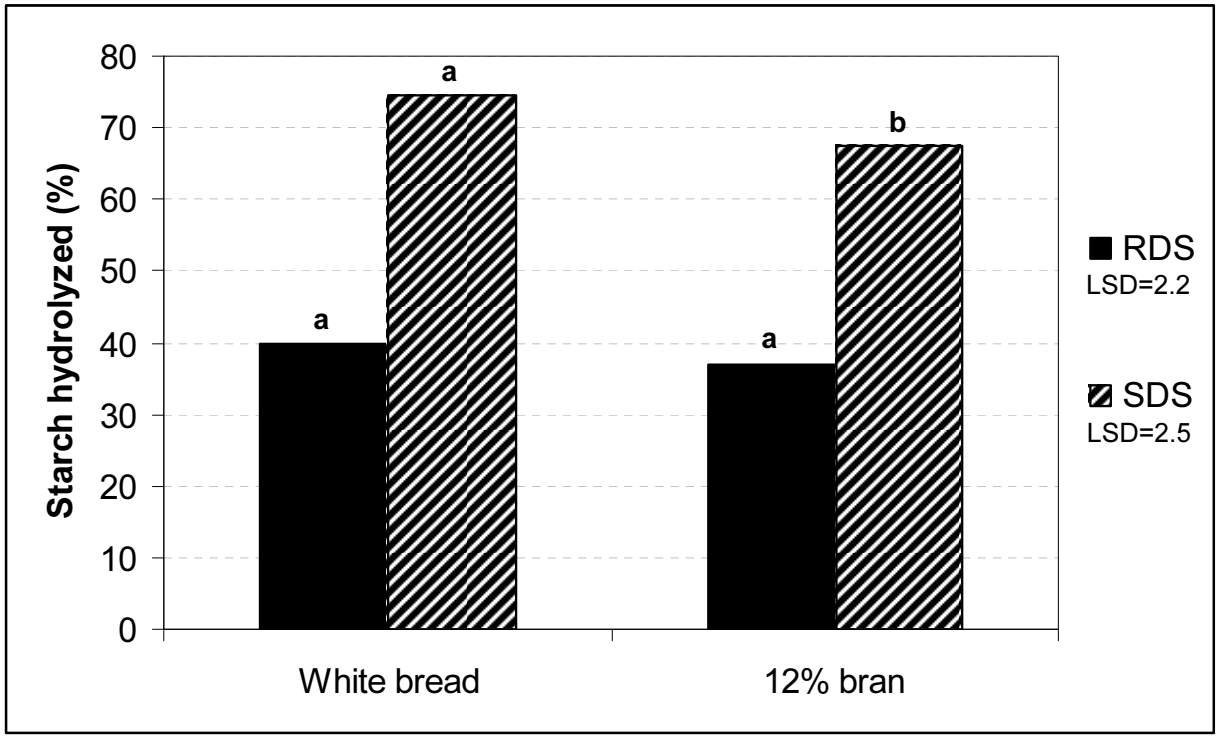


**Fig. 26. Rapidly digestible starch (RDS), and slowly digestible starch (SDS) of breads (dry wt basis). Treatments with the same letter within each type of bar are not significantly different ($p<0.05$).**

**Table 20. Hydrolysis Index (HI) and estimated Glycemic Index (EGI) of breads.**

| | HI | EGI |
|---|---|---|
| White bread | 91 ±5 a | 90 ±3 a |
| Bread 12% sorghum bran | 80 ±3 b | 84 ±2 b |
| LSD | 5.5 | 3.0 |

**Table 21. Glycemic index (GI) of some white breads and high-fiber breads reported in the literature (from in vivo studies).**

| Product | GI* |
|---|---|
| White Turkish bread | 124 |
| Wonderwhite (White resistant starch-enriched bread, Buttercup Bakeries, Australia) | 114 |
| Fiber white (White resistant starch-enriched bread, Nature's Fresh) | 110 |
| Gluten free, fiber-enriched bread (gluten-free wheat starch and soy bran) | 104±5 |
| White-wheat flour bread (Canada) | 102±5 |
| Wonder, enriched white bread | 101±13 |
| White-wheat flour bread (USA) | 100 |
| White, high fiber bread (Weton's Bakery, Canada) | 98±5 |
| White fiber-enriched bread (Dempster's Corporate Foods, Ltd, Canada) | 96±6 |
| White bread + 15 g psyllium (*Plantago psyllium*) | 76±17 |
| Bread with 45% oat bran | 72±10 |

From Foster-Powell et al. 2002.
* Reference food: white bread (GI=100).
Note: When glucose is used as a reference food, (GI of glucose=100, GI of bread=70), the GI value of the food is divided by 0.7 to obtain the GI value with white bread as a reference food.

bread (Table 20). Other studies have reported a reduction in the GI value when bran or fibers are added to breads (Brown 2004, Foster-Powell 2002).

In this study, the EGI of the wheat bread was 90. While it is common to use referenced and experimental GI values of white bread as 100, wheat bread has shown different GI values due to differences in the type of flour, formulation, and preparation procedures (Table 21). EGI values of bread with added tannin bran is compared with the GI of breads containing added fibers (Table 21). Tannin sorghum bran has potential to reduce EGI of bread significantly.

# CHAPTER VI
# SUMMARY AND CONCLUSIONS

## Summary

Fractions from white and tannin sorghums were processed into extrudates, porridges, and breads. For each preparation treatment, the starch availability of the sorghum products was affected by the processing conditions used (Table 22).

**Table 22. Type of products, grain fractions, and general processing conditions.**

| | **Extrudates** | **Porridges** | **Breads** |
|---|---|---|---|
| **Sorghum fractions** | Whole, cracked, and decorticated grains | Whole and decorticated flours | Tannin bran (12% addition) |
| **Processing conditions** | | | |
| Temperature | Very high | High | Very high |
| Time | Very short | Short | Long |
| Moisture | Low | High | High |
| Pressure | High | Normal | Normal |

### *White sorghum products*

White sorghum products were more slowly digested than corn products for both processing treatments (extrudates and porridges) (Fig. 27). When the same fractions were compared (whole and decorticated), digestibility rates of the extrudates were higher than the porridges, as well as their EGI (Table 23).

A higher degree of starch gelatinization (increasing the susceptibility of starch to enzyme hydrolysis) was obtained from extrusion processing compared to porridge making. A sorghum with a hard endosperm is preferred for extrusion processing to form a complete gelatinized melted matrix that is fully expanded. In the thick porridge, a hard endosperm is also desired to obtain good textural quality, however, proteins from the corneous endosperm appear to limit the extent of starch gelatinization.

The EGI of the decorticated white sorghum products were higher than the whole-grain products. The EGI of a decorticated extrudate (102) and a decorticated porridge (98), were higher than a whole grain extrudate (92) and whole grain porridge (87) respectively (Table 23). In the white sorghum products, the grain fraction used has a major effect on the starch digestibility and EGI, besides the type of processing.

***Tannin sorghum products***

Products with tannin sorghum fractions were more slowly digested than corn (extrudates and porridges) and wheat (bread). However, the effect of processing on the tannin sorghum products was different from the one seen in the white sorghum products. When the same fractions were compared, digestibility rates of the tannin porridges were higher than the tannin extrudates (Fig. 28). Therefore, a higher degree of starch gelatinization was obtained from the porridge making compared to extrusion processing. The soft endosperm nature of tannin sorghum is one of the main factors affecting starch gelatinization. During extrusion, a soft endosperm was detrimental, because not enough friction was generated inside the extruder to form a fully gelatinized melted matrix. On the other hand, in porridge making, a soft and fine flour was easy to gelatinize, because of the loose packing of the starch granules in the protein matrix.

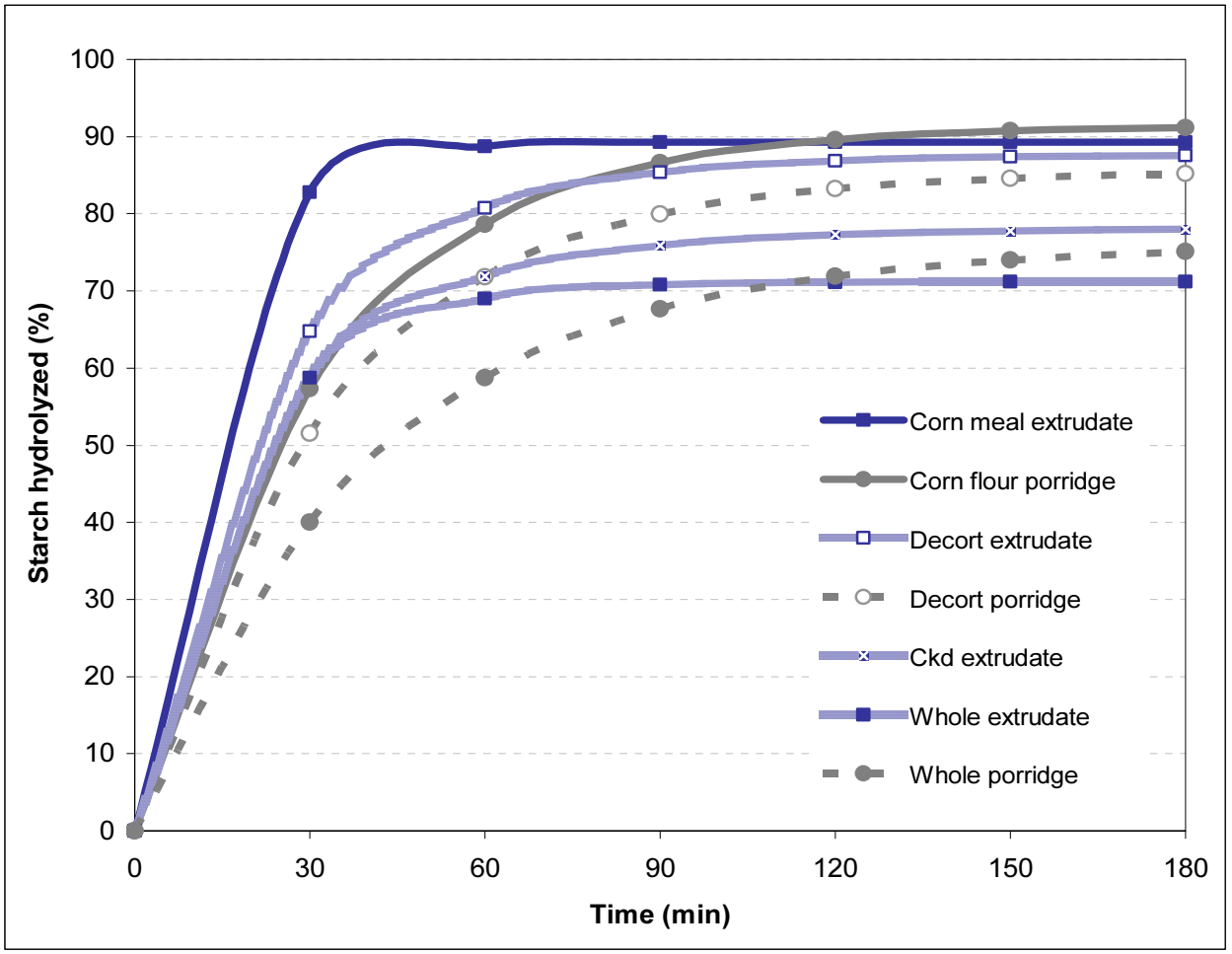


**Fig. 27. Starch digestibility of white sorghum products compared to corn products.**

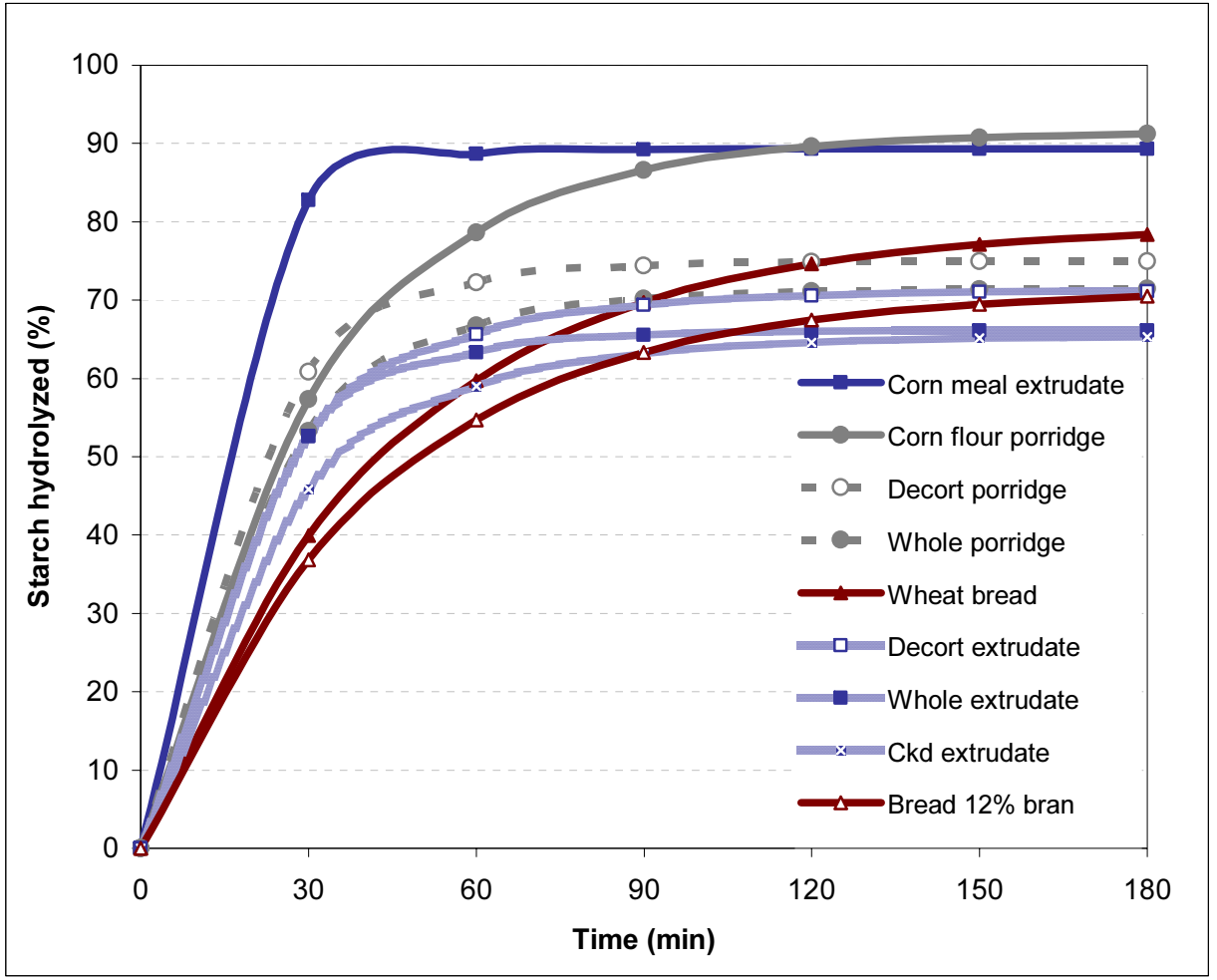


**Fig. 28. Starch digestibility of tannin sorghum products compared to corn/wheat products.**

**Table 23. Estimated glycemic index (EGI) of food products (mean values).**

| Grains and products | EGI |
|---|---|
| **Corn** | |
| Corn meal extrudate | 108 |
| Corn flour porridge | 101 |
| **White sorghum** | |
| Decort extrudate | 102 |
| Decort porridge | 98 |
| Ckd extrudate | 96 |
| Whole extrudate | 92 |
| Whole porridge | 87 |
| **Tannin sorghum** | |
| Decort porridge | 95 |
| Whole porridge | 91 |
| Decort extrudate | 90 |
| Whole extrudate | 88 |
| Ckd extrudate | 85 |
| Bread 13% tannin bran | 84 |
| **Wheat** | |
| Wheat bread | 90 |

Processing facilitated associations of condensed tannins with starch and protein affecting starch gelatinization and starch available for digestion.

The EGI of the decorticated (95) and whole grain tannin sorghum porridges (91) were followed by the decorticated (90) and whole grain (88) tannin extrudates, and bread with 12% of the tannin bran fraction (84) (Table 23). In the tannin sorghum products, besides the fraction used, the type of processing has a major effect on the starch digestibility and EGI of the products.

***Estimation of glycemic index***

In the present study, the HI of each sample was used to obtain the EGI, however, alternatively to using the HI value, Goñi et al. (1997) proposed using the hydrolysis value of 90 min to estimate the GI value of foods based on a correlation found with the in vivo glycemic response ($r=0.909$, $p<0.05$). Using all the samples from the present study, a high linear correlation was found between the HI value and both the hydrolysis values at 60 and 90 minutes (Table 24, Fig. 29). This confirms the suggestion by Goñi et al. (1996) that the calculation of the GI could be simplified by using just one point of the hydrolytic process to estimate the GI (less experimental time). From the results of this study, the percentage of starch hydrolyzed at 60 min is a potential alternative value to estimate GI of sorghum products. However, in vivo glycemic measurements should be performed to verify a correlation, and an equation should be further developed to obtain the EGI using the hydrolysis value of 60 min.

**Conclusions**

In vitro starch digestibilities and EGI of sorghum products were significantly lower than corn products (extrudates and porridges). Differences in grain composition and characteristics in white and tannin sorghums affected the rate of starch digestion. The tannin sorghum products had a lower EGI compared to white sorghum products. Furthermore, the addition of tannin sorghum bran reduced the EGI of wheat bread significantly. These results have important implications for the processing of sorghum for food use.

**Table 24. Correlation between the percentage of starch hydrolysis at different sampling times and the calculated hydrolysis index.**

| Sampling time (min) | $R^2$ value ($p<0.05$) |
|---|---|
| 30 | 0.70 |
| 60 | 0.95 |
| 90 | 0.94 |
| 120 | 0.83 |
| 150 | 0.76 |
| 180 | 0.72 |

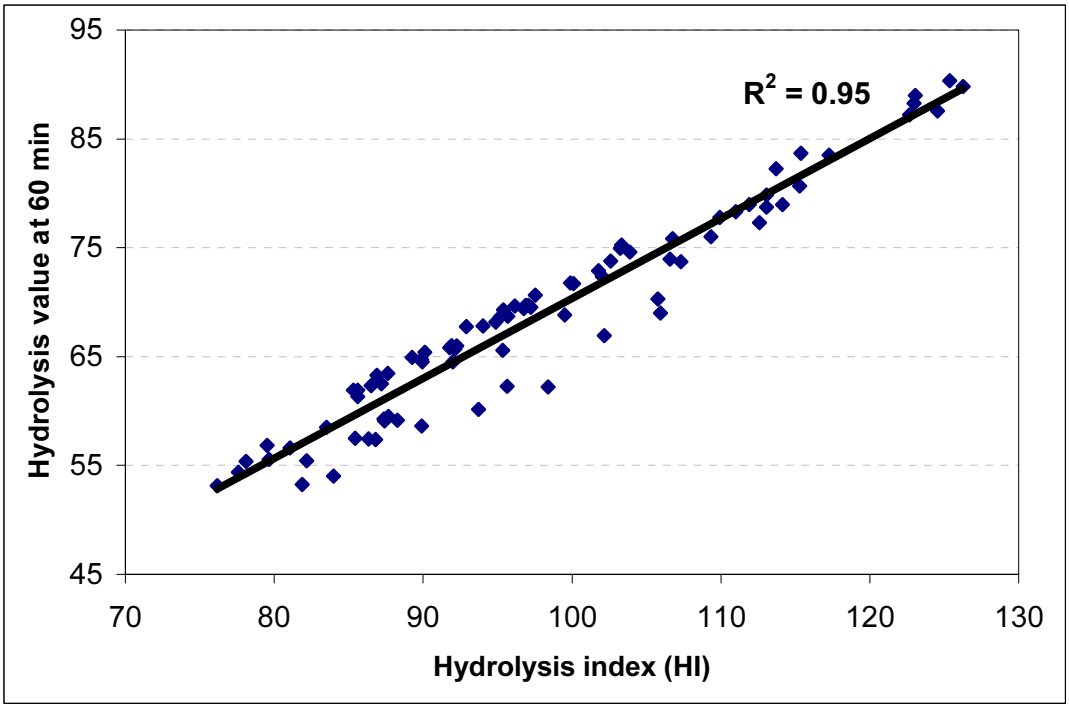


**Fig. 29. Linear regression line and correlation between starch hydrolyzed at 60 minutes and hydrolysis index.**

The selection of specialty sorghum cultivars (e.g. high-tannin varieties), as well as adaptation of processing conditions may offer possibilities for the development of food products with reduced glycemic response. The findings from this work need to be further consolidated through in vivo measurements of glycemic index.

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# APPENDIX A
# EFFECT OF PARTICLE SIZE ON STARCH AVAILABILITY OF CORN PORRIDGES


**Fig. A-1. Raw materials used to prepare corn porridges.**

**Table A-1. Particle size distribution of corn meal vs. corn flour.**

| | #40 (425 µm) | #60 (250 µm) | #80 (180 µm) | #100 (150 µm) | Plate (<150 µm) |
|---|---|---|---|---|---|
| Corn meal | 99.2 | 0.3 | 0.2 | 0.1 | 0.2 |
| Corn flour | 20.9 | 51.1 | 24.7 | 2.4 | 1.2 |

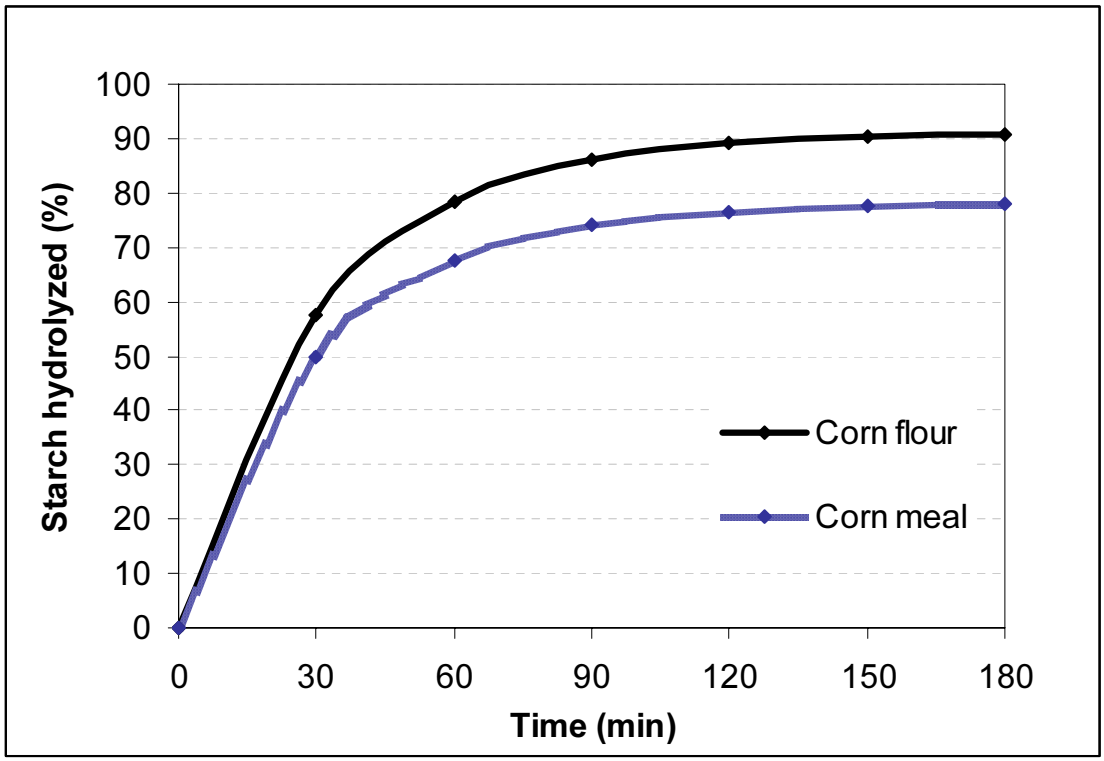


**Fig. A-2. In vitro starch digestibility of corn porridges from 0 to 180 min.**

**Table A-2. Percentage (dry wt basis) of starch hydrolyzed at different times (min), and calculated C∞ and k constants for each type of corn porridge.**

| Type of porridge | Time (min) | | | | | | C∞ | k |
|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | | |
| Corn meal | 49.7 | 67.7 | 74.2 | 76.6 | 77.5 | 77.8 | 78.0 | 0.03 |
| Corn flour | 57.5 | 78.5 | 86.3 | 89.2 | 90.2 | 90.7 | 91.5 | 0.03 |

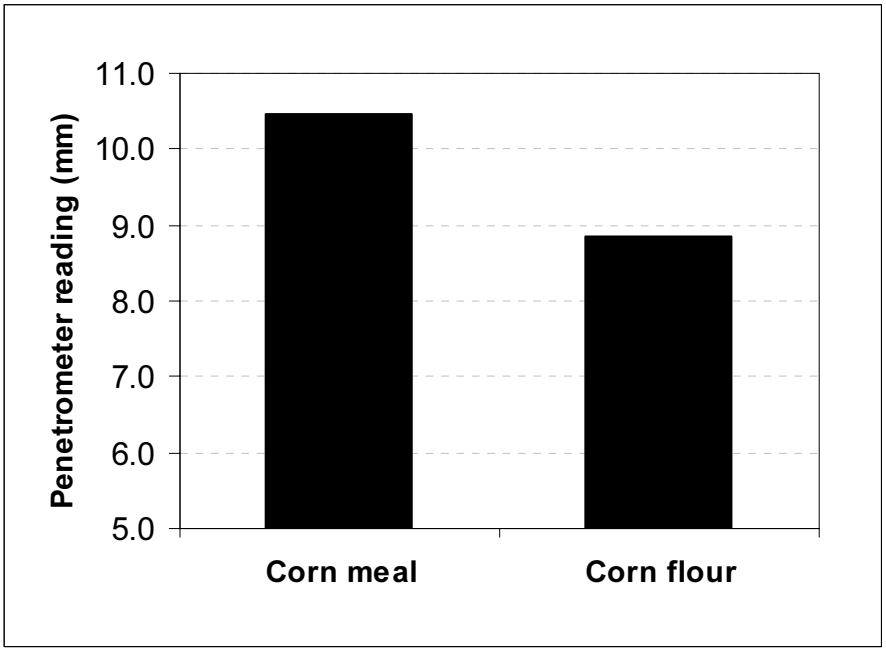


**Fig. A-3. Texture quality (softness) of fresh corn porridges based on penetrometer readings.**

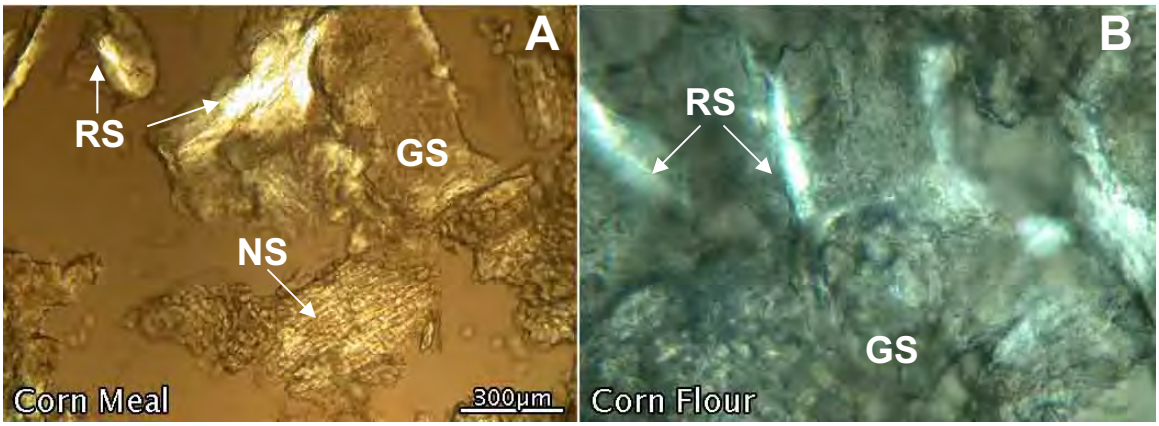


**Fig. A-4. Corn porridges seen under light microscopy. A: Corn meal porridge, B: Corn flour porridge (GS: gelatinized starch, RS: Retrograded starch, NS: native starch).**

# VITA

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