THE EFFECTS OF SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] PHENOLIC COMPOUNDS ON STARCH DIGESTIBILITY OF PORRIDGES

A Dissertation

by

DILEK AUSTIN

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

DOCTOR OF PHILOSOPHY

December 2008

Major Subject: Food Science and Technology

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Approved by:

Chair of Committee,	Lloyd W. Rooney
Committee Members,	Luis Cisneros-Zevallos
	Mian N. Riaz
	Nancy D. Turner
Chair of Food Science and Technology Faculty,	Jimmy Keeton

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ABSTRACT

The Effects of Sorghum [*Sorghum Bicolor* (L.) Moench] Phenolic Compounds on Starch Digestibility of Porridges. (December 2008)

Dilek Austin, B.Sc., Ankara University;

M.Sc., Ankara University;

M.Sc., University of Nebraska-Lincoln

Chair of Advisory Committee: Dr. Lloyd W. Rooney

Two tannin sorghums, (high-tannin, black with tannin), high anthocyanin sorghum (black), and non-tannin sorghum (white) were used to investigate the effects of sorghum phenolic compounds on *in vitro* starch digestibility, estimated glycemic indices (EGI) and resistant starch contents (RS) of porridges. Sorghum varieties were chosen to have a wide range of total phenols (3-23 mg/g gallic acid) and tannin contents (0-34 mg catechin eq./g). Normal corn starch, enzyme resistant high amylose corn starch, and whole sorghum grains were cooked with the aqueous fraction of sorghum bran extracts obtained with 70% aqueous acetone. Endosperms of soft and hard sorghum varieties were mixed with sorghum brans and cooked into porridges with distilled water.

Hi-tannin, black and black with tannin sorghum bran extracts significantly ($p<0.05$) decreased starch digestibility and estimated glycemic index (EGI) while they increased resistant starch (RS) contents of normal corn starch, enzyme resistant high amylose corn starch, and whole sorghum grain porridges. The highest reduction in starch digestibility of the porridges occurred with hi-tannin sorghum bran extracts, followed by black with tannin and black sorghum bran extracts. Double cooked corn starch porridges, which were cooked with these bran extracts had EGI values of 49-67 and RS contents of 9.1-57.7%. These RS values are higher than foods such as legumes, whole

pasta and whole grain cereals which are considered health foods with low EGI (36-71) and high RS contents (2.9-6.8). Only brans of condensed tannin-containing sorghum varieties (tannin, black with tannin sorghums) significantly ($p<0.05$) decreased starch digestibility, and EGI, and increased RS contents of the endosperm porridges. When tannin sorghum bran extracts were cooked with zein added to corn starch porridges, starch digestibility of the porridges significantly ($p<0.05$) increased, while RS significantly ($p<0.05$) decreased because the zein reacted preferentially with the tannins.

The cooking trials indicated that sorghums with tannins significantly reduced the activity of digestive enzymes, reduced EGI, and increased RS contents of porridges. Thus, specialty sorghum varieties have a potential to lower EGI and increase RS contents of starchy foods. Their aqueous bran extracts have potential use to reduce risk factors for type II diabetes and obesity.

DEDICATION

To my husband: my rock, and inspiration,

To my son: my sunshine,

To my daughter: my butterfly.

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I would like to thank my supervisors, Dr. Lloyd W. Rooney, and the late Dr Ralph D. Waniska, for making all this work possible and giving me the opportunity to work with them, I feel honored and humbled by the experience and insight that they have shared with me during my program. I am also indebted to Drs. Nancy D. Turner, Mian N. Riaz and Luis Cisneros-Zevallos for serving on my doctoral graduate committee and for their helpful advice and suggestions.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

CHAPTER I

INTRODUCTION

Sorghum bicolor (L.) Moench is the fifth most important cereal crop after wheat, rice, maize, and barley in terms of production (FAO 2005). Drought tolerant sorghum is a very important dietary component in many parts of Africa, Asia, and the semi-arid tropics worldwide (Serna-Saldivar and Rooney 1995).

Some specialty sorghum varieties are less digestible than the other cereals (Rooney and Pflugfelder 1986). They also contain substantial levels of a wide variety of phenolic compounds, such as phenolic acids, anthocyanins, and condensed tannins. A previous study by De Castro Palomino Siller (2006) showed that tannin sorghum extrudates and porridges had reduced starch digestibility and EGI, and increased resistant starch (RS) values compared to corn extrudates and porridges. Among specialty sorghum varieties, tannin sorghum digestibility has been studied the most (Taylor et al 2007, Oliveira et al 2007, De Castro Palomino Siller 2006, Mariscal-Landín et al 2004, Matuschek and Svanberg 2004, Nyamambi et al 2000); there is little published information about the other specialty sorghum varieties, such as anthocyanin-rich black sorghum.

Because obesity and diabetes are among the most important medical problems in America today, investigation of starch digestibility, EGI, and RS in the presence of sorghum phenolics, specifically condensed tannins and anthocyanins, would be useful.

Postprandial blood glucose changes can be used to categorize the Glycemic Index (GI). GI is a scale that ranks carbohydrate-rich foods by how much and how quickly they raise blood glucose levels compared to a standard food (glucose or white bread). GI can be estimated by *in vitro* rate and extent of starch digestibility, which is called Estimated Glycemic Index (EGI) (O'Dea and Holm 1985, Lund and Johnson 1991).

This dissertation follows the style and format of Cereal Chemistry.

Starch escapes from digestion in the small intestine (may be digested in the large intestine), does not cause a glycemic response This type of starch is called resistant starch (RS).

Therefore, the objectives of this study were:

- To characterize physical and chemical characteristics of the sorghum varieties with different amounts of phenolic compounds,
- To determine the effects of these sorghum brans on starch digestibility of soft and hard sorghum endosperm porridges,
- To investigate the effects of these sorghum bran extracts, which contains condensed tannins and anthocyanins, on starch digestibility, and EGI, and RS of corn starch, enzyme resistant high amylose corn starch and whole sorghum porridges.

CHAPTER II

LITERATURE REVIEW

Sorghum

Sorghum is classified as sorghum, tannin sorghum, white sorghum, and mixed sorghum (FGIS-GIPSA 2006). Sorghum is most often consumed as porridge in many countries. Porridge preparation involves cooking the meal with boiling water; the process varies considerably depending on the type of porridge being produced (Bello et al 1990).

Sorghum Kernel Structure

The grain's principal anatomical components are the pericarp (outer layer), testa or seed coat, the endosperm tissue and the germ (embryo and scutellum) (Rooney and Sullins 1973).

Endosperm

The endosperm tissue of sorghum, like other cereals, is composed of the aleurone layer, peripheral, corneous and floury areas. The aleurone is the outer layer of the endosperm and consists of a single layer of rectangular cells with thick cell walls (Rooney and Sullins 1973, Rooney and Miller 1982). The peripheral endosperm is extremely dense, hard, and resistant to water penetration, and digestion. Corneous (hard) endosperm has a continuous protein matrix that contains protein bodies dispersed throughout, with the starch physically surrounded by the protein. Floury (soft) endosperm in the center of the granule has a discontinuous protein matrix and few protein bodies (Ring et al 1988).

Pericarp

The pericarp of sorghum grain originates from the ovary wall and is divided into three layers, the epicarp, mesocarp, and endocarp, with a seed coat lying underneath. Unlike other cereals, the mesocarp of some sorghum varieties contains starch granules (Rooney and Waniska 2000).

The color of the sorghum pericarp appears to be due to a combination of primarily anthocyanin and anthocyanidin pigments and other flavonoid compounds in the pericarp (Hahn et al 1984). Some sorghum varieties have a pigmented seed coat (testa) that contains condensed tannins (Earp and Rooney 1986, Earp et al 2004).

Sorghum Starch

Sorghum Starch Digestibility

The starch granules of normal corn and sorghum are very similar in size (5-20 μm), shape and composition. The major differences between corn and sorghum are the type and distribution of proteins surrounding the starch in the endosperm (Rooney and Waniska 2000).

Sorghum starch is digestible and biologically equivalent to maize starch (Rooney and Riggs 1971, Hale 1973). Experience with livestock feeding (Riley 1984) and brewing (Goode and Arendt 2003) suggests that starch in whole sorghum grain may be slightly less digestible due to the hard peripheral endosperm layer limiting access to the interior (Rooney and Pflugfelder 1986, Hamaker and Bugusu 2003). Processing methods such as steam-flaking and reconstitution are effective in raising sorghum digestibility to that of corn by breaking open the kernels and exposing the interior (Rooney and Pflugfelder 1986). Previous *in vitro* studies by De Castro Palomino Siller (2006) indicated that condensed tannins in the tannin sorghum may have interfered with starch digestion after 60 min of incubation, because tannin sorghum porridges reached a steady state of starch digestion, while white sorghum porridges had a higher percentage of starch digested.

Resistant Starch (RS)

Starch that is not digested in the human small intestine and enters the large intestine is called RS (Englyst et al 1992). The presence of RS has been associated with the physical entrapment of starch within whole or partly milled grains or seeds (RS1), ungelatinized granules of B-type starches (RS2), and starch retrogradation during food processing (RS3) (Englyst et al 1992, Englyst and Cummings 1985, Tester et al 2004, Gordon et al 1997).

Phenolic Compounds

Phenolic compounds exist as free aglycones or as conjugates with sugars (Asquith et al 1987, Price and Butler 1977). In sorghum, the phenolic compounds are generally located in the outer layers of the kernel in the epicarp and testa layers (Awika and Rooney 2004, Earp et al 2004). Phenolic compounds in sorghum can be divided into three major categories: phenolic acids, flavonoids and condensed tannins (Hahn et al 1984). All sorghums contain phenolic compounds, and the amount of phenolic compounds is influenced by both genotype and the environment (Dykes et al 2005).

Condensed Tannins

Condensed tannins are dimers, oligomers, and polymers of catechins that are bound together by links between C4 and C8 (or C6) (Hahn et al 1984). In tannin sorghum, the testa is pigmented and this feature is controlled by the complementary B_1 and B_2 genes (Ring et al 1988). The spreader gene (S) controls the presence of pigments and possibly tannins in the epicarp. When S is dominant, more tannins are in the pericarp and testa layers. Type II sorghums have a pigmented testa and recessive spreader gene (B_1- B_2- ss), while type III sorghums have a pigmented testa and dominant spreader gene (B_1- B_2- S-). Type I sorghums do not have a pigmented testa and do not contain tannins (Hahn et al 1984, Dykes et al 2005).

Although tannins are considered anti-nutritional compounds, tannin sorghums have been consumed and preferred for centuries as breads, porridges, and alcoholic beverages in Africa and Asia. Awika and Rooney (2004) stated that tannin sorghums can and should be considered as a source of natural antioxidants, dietary fiber, and color compounds. De Castro Palomino Siller (2006) reported that the addition of 12% tannin bran to bread formulation significantly ($p<0.05$) decreased starch digestibility and EGI values. McDonough et al (2004) reported that tannin sorghum bran resisted oxidative damage due to high-energy irradiation.

Anthocyanins

Anthocyanins are naturally occurring, water-soluble compounds. The most abundant anthocyanins in sorghum grain are 3-deoxyanthocyanidins, e.g. apigeninidin

and luteolinidin (Dicko et al 2006). Glycosylated forms of these anthocyanins were also identified in sorghums by Nip and Burns (1969, 1971). Black sorghum has significantly ($p<0.05$) more anthocyanin pigments than other sorghums (Awika and Rooney 2004). Awika and Rooney (2004) reported that black sorghum contained 4-9.8 mg/g anthocyanins, followed by red sorghum (3.3 mg/g), and brown sorghum (1.6-3.9 mg/g) on a dry weight basis.

In vivo studies showed that anthocyanins may help prevent obesity (Prior et al 2007), diabetes (Jankowski et al 2000), heart disease (Tsuda et al 2003), inflammation (Lietti et al 1976), and certain cancers (Karaivanova et al 1990, Turner et al 2006). Jankowski et al (2000) reported that grape anthocyanins prevented the generation of free radicals, decreased lipid peroxidation, reduced pancreatic swelling, and decreased blood sugar concentrations in urine and blood serum. Purple corn anthocyanins inhibited the typical symptoms of hyperinsulinemia, and hyperleptinemia, which generally occur with high-fat diets (Tsuda et al 2003).

Phenolic Compounds and Starch Digestibility

Phenolic compounds complex with proteins (Haslam 1996; Riedl and Hagerman 2001) and carbohydrates (Asquith et al 1987; Naczk et al 2006), generating insoluble compounds. These interactions with phenolics include hydrophobic associations in nature and the formation of hydrogen bridges through hydroxyl groups (Belitz and Grosch 1999, Juge and Svensson 2006). Tannins bind sorghum kafirin (prolamine rich protein) resulting in reduced protein levels, and consequently lower and slower starch digestibility of tannin sorghum.

Davis and Hoseney (1979) reported that tannins isolated from sorghum grain inhibited the enzyme alpha-amylase, and they also bind to the starch granule to varying degrees. Daiber (1975) and Beta et al (2000) found that condensed tannins inactivated malt amylases, which reduced starch breakdown and sugar production during brewing. De Castro Palomino Siller (2006) stated that condensed tannins inhibit the starch digestion by limiting starch availability in tannin sorghum extrudates and porridges.

Thompson and Yoon (1984) investigated the relationship between polyphenol

intake and the blood glucose response in healthy and diabetic volunteers. They found a negative correlation between the GI and the concentration/total intake of polyphenols. Polyphenols, especially the large polymeric type or condensed tannins, appear to be responsible in part for the reduced glycemic response to carbohydrate foods and relatively low blood glucose response to legumes compared with cereal products (Thompson and Yoon 1984).

Phenolic compounds complexing with starch and inhibiting enzymes may also lead to an increase in RS amount in porridges. De Castro Palomino Siller (2006) found that tannin sorghum porridges had significantly higher RS content than white sorghum porridges. Dicko et al (2006) reported that cooling cooked porridge led to the formation of RS which may form complexes with kafirin proteins; these are less susceptible to enzyme attack, resulting in increased RS in porridges.

Glycemic Index (GI) and Estimated Glycemic Index (EGI)

The glycemic index (GI) ranks carbohydrate-containing foods on how quickly and how much they elevate blood sugar levels. It is measured by comparing the increase in blood sugar after eating 50 grams of available carbohydrate from a single food with the increase in blood sugar after eating the same quantity of available carbohydrate from a reference food, which is either glucose or white bread (Jenkins et al 1981). A food that is easily broken down during digestion and quickly absorbed has a fast and high blood glucose response. Foods can be classified as having a low (<55), intermediate (55-70), or high GI (>70) with glucose as the reference standard. Higher values are generally found in more highly processed foods, while whole grain foods have lower values (Wolever and Bolognesi 1996). Consuming low GI foods helps to prevent extreme blood glucose changes and to slow absorption of carbohydrates (Price and Butler 1977, Jarvi et al 1999). This is helpful for people with diabetes and beneficial in preventing certain diseases (Price and Butler 1977).

The GI can be estimated from *in vitro* starch digestibility, and is called Estimated Glycemic Index (EGI). The rate and extent of starch digestibility depends on intrinsic and extrinsic factors, like cooking method (gelatinized starch is more easily digested);

processing method (reduced particle size; fiber and protein removal make digestion easier); type of starch (amylopectin is more easily digested than amylose); fiber (viscous soluble fiber slows digestion); fat (slows digestion and absorption of food components), inhibitors (tannins), and acidity (slows stomach emptying and the digestion rate) (Pi-Sunyer 2002).

CHAPTER III

MATERIALS AND METHODS

Sorghum Varieties and Physical Characterization

Sorghum Varieties

White food-type sorghum (tannin free, ATx635*RTx436, College Station, 2003), sumac sorghum (tannin sorghum, West Texas, 2003), hi-tannin sorghum (tannin sorghum, CSC3xR28, College Station, 2001), black sorghum (tannin free with anthocyanin, TX430 black, College Station, 2001), and black with tannin sorghum (tannin-anthocyanin, Black PI Tall, College Station, 2005) varieties were used for this study. Sorghum grains were cleaned, de-glumed, and stored at 4°C until needed.

Grain Characterization

Sorghum grain was characterized for hardness (hardness index, HI), thickness (diameter) and weight with a single kernel hardness tester (SKHT, model SKCS 4100, Perten Instruments, Reno, NV). The instrument gives mean data based on 300 kernels. Density was measured using a gas-comparison pycnometer (Multipycnometer, Quantachrome, Syosset, NY). Five kernels of each type of sorghum were dissected and endosperm appearance was evaluated visually. Thousand-kernel weight (TKW) was performed by weighing 40 kernels and multiplying by 25. Color (L) lightness; +a) red, -a) green; +b) yellow, -b) blue of whole sorghum grains and whole sorghum flour was measured using a colorimeter (model CR-310 Minolta Co. Ltd., Ramsey, NJ). Analyses were conducted in quadruplicate. Particle size distribution of the sorghum brans 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.

Controls Used in the Study

- A white corn variety (Cargill Inc, Minneapolis, MN),
- A white food-type sorghum variety (ATx635*RTx436, College Station, 2003),
- A commercial corn starch (Argo)

- High amylose corn starch (High-maize starch 260, National Starch & Chemical, Bridge Water, NY) were used as controls.

They were kept at –20 ^{0}C until used.

Milling and Bran Extract Preparation

Whole Sorghum Grain Flours

Whole sorghum grains were milled to pass through a 1 mm screen using a UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO). They were kept at –20 ^{0}C until used.

Pure Sorghum Endosperm

According to Hardness Index values (HI), sumac sorghum was relatively softer, while white sorghum variety was relatively harder than the other sorghum varieties. Sumac sorghum variety (soft endosperm), and white sorghum variety (hard endosperm) were decorticated in an abrasive mill until 30% of the original kernel weight was removed. These endosperms were free of bran and germ. Endosperms and brans were separately milled through 1 mm screen (Model 3010-030, Udy Corporation, Fort Collins, CO).

Sorghum Brans

Brans of sorghum varieties were obtained by decorticating 4-kg batches in a PRL mini-dehuller (Nutama Machine Co., Saskatoon, Canada). The bran was then separated with a KICE grain cleaner (Model 6DT4-1, KICE Industries Inc., Wichita, KS). The bran (approx.10% of original grain weight) was further milled to pass through a 1 mm screen using a UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO). They were kept at –20 ^{0}C until used.

Sorghum Bran Extracts

Distilled water, absolute methanol, 70% aqueous acetone, and 70% aqueous ethanol were used for extractions of sorghum phenols. The milled brans (3 g) were extracted for 3 hr using 30 ml of solvent with constant shaking at low speed in an Eberbach shaker (Eberbach Corp., Ann Arbor. MI) in quadruplicate (4x3g=12 g bran; 30X4=120 ml solvent). The extracts were centrifuged, and supernatants (~100 ml) were

collected. Solvents (70% aqueous acetone, and 70% aqueous ethanol) were removed with vacuum at 30 ^{0}C, and phenols were recovered in approximately 30 ml of water, which were used for making porridges. The extracts of absolute methanol were evaporated to dryness at 25 ^{0}C in a Speed Vac SC201A (Thermo, Marietta, OH) under vacuum. The dried residue was dissolved in 25.1 ml water, and used for porridge making. Extracts were kept at –20 ^{0}C until the next day. Extracts were brought to room temperature to make porridges.

Solid Contents of Extracts

Solid contents of bran extracts were measured after acetone removal as described above with a Portable Digital Brix Refractometer (Model ATA-3810 PAL-1, Pulse Instruments, Van Nuys, CA).

Sorghum Bran Residues After 70% Aqueous Acetone Extraction

After removal of extractable sorghum phenolics from brans with 70% aqueous acetone, brans were placed in an air forced oven at 35 ^{0}C overnight. Dried bran residues were further milled to pass through a 1 mm screen using a UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO). Residues were kept at –20 ^{0}C until used. Residues were brought to room temperature to make porridges.

Chemical Characterization

Proximate Composition of the Grain

Moisture

Moisture content of sorghum grains were determined by the moisture air oven method (AACC 2000, Method 44-19) in triplicate.

Total Starch

Total starch (TS) was determined by AACC 2000, Method 76.13 using the Total Starch Assay Procedure Kit (Megazyme Int, Ireland). A 100 mg of 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.

Protein Contents

Protein contents of the whole grains were determined with a Perten PDA 7000 NIR (Perten Instruments, Reno, NV).

Total Phenol and Tannin Content Analysis

Whole sorghum grain, sorghum brans (10% decortication), and bran residues were analyzed for total phenol and tannin contents. The milled samples were extracted by shaking at low speed in an Eberbach shaker (Eberbach Corp., Ann Arbor. MI) using acidified methanol (1% HCl in methanol) for whole sorghum grain; 70% aqueous acetone for sorghum brans and bran residues. The tubes were centrifuged at 3000 rpm for 10 minutes using the Beckman Model TJ-6 centrifuge (Beckman Instruments Inc., Spinco Division, Palo Alto, CA). The supernatants were used for total phenol and tannin quantifications. The reason for using 70% aqueous acetone for brans and bran residues to extract phenols was to be consistent with bran extracts which were added in porridges.

Total Phenol Content Analysis

The Folin Ciocalteu method of Kaluza et al (1980), as modified by Dykes et al (2005), was used to determine total phenols of sorghum varieties. The milled samples were extracted for 2 hours at low speed in an Eberbach shaker (Eberbach Corp., Ann Arbor. MI). One aliquot of the supernatant (0.1 ml) was diluted with 1.1 ml of water and was then reacted with 0.4 ml of Folin reagent and 0.9 ml of 0.5 M ethanolamine. The reaction was allowed to stand for 20 min at room temperature, and the absorbance was read at 600 nm. Gallic acid was used as a standard.

Tannin Content Analysis

The vanillin-HCl method of Price et al (1978), and modified by Dykes et al (2005) was used to determine tannin contents of sorghum varieties. The milled samples were extracted at 30^0 C for 20 min. A 1 mL volume of the supernatant was mixed with 5 mL vanillin reagent, and absorbance read at 500 nm after 20 min. Blank determinations were done to compensate for the color of the samples, by replacing the vanillin reagent with 4% HCl in methanol. The standard used was catechin (Sigma-Aldrich Inc., St Louis, MO); tannin content was expressed as mg catechin equivalents per g (mg CE/g).

Porridge Preparation

A Rapid Visco Analyzer (RVA) (RVA Series 4, Newport Scientific, Warriewood, Australia) was used to cook porridges. A programmed heating and cooling cycle was used, in which the mixture was held at 50^0 C for 1min, heated to 95^0 C in 7.5 min at the rate of 6^0C/min, held at 95^0 C for 5min before cooling to 50^0 C in 7.5 min and holding at 50^0 C for 1min (Appendix C). Porridges were left at room temperature for 10 min before sub-sampling for enzyme hydrolysis. Solid-to-liquid ratios of porridges were between 11-13% db. The viscosity was recorded (RVA Series 4) with the accompanying software Thermocline for Windows Version 2.0.

Porridges Used in Preliminary Studies

Whole sorghum porridges were prepared with 2.8 g (dry basis) starch equivalent ground whole sorghum and 25.1 ml distilled water.

The effects of tannin bran extracts on corn starch porridges were observed before cooking and after cooking. Tannin bran extracts (25.1 ml) were added to corn starch porridges during cooking and 10 min after cooking. The mixture was homogenized using a Polytron homogenizer (Kinematica GmbH, Switzerland) with controlled speed (level 4) for 1 min.

Porridges Used in the Main Study

Endosperm Porridges Cooked with Sorghum Brans

Endosperms of hard (white sorghum) and soft (sumac sorghum) sorghum varieties were mixed with sorghum brans (endosperm: bran ratio of 85:15), and cooked in 25.1 ml of double distilled water using Rapid Visco-analyzer (RVA Series 4) for 7 min. Endosperm porridges cooked with white bran and hard/soft endosperm porridges cooked without any brans were chosen as controls.

Double Cooked Corn Starch Porridges

Single cooked corn starch (3.1 g, as is) with 25.1 ml distilled water was used as a control. Double cooking-cooling cycle was applied with different amounts of distilled water as follows;

Double Cooked Corn Starch Porridges: Porridges were cooked with 3.1 g (as is) corn starch and 25.1 ml distilled water, cooled to room temperature, and cooked again.

Double Cooked Corn Starch Porridges wit Limited Water: At first cooking, corn starch (3.1 g as, is) was cooked with 12 ml of distilled water, and then cooled to room temperature for 10 min. Thirteen ml (13.1 ml) additional distilled water was added to the cooked porridge for the second cooking (12+13.1=25.1 ml distilled water).

All porridges mentioned below were double cooked as described in #2 above. The amounts of bran extracts used in porridges mentioned below were the entire amounts of extracts obtained from 4 centrifuge tubes of 3 g sorghum bran (4X3=12 g bran) and approximately 30 ml distilled water (4X30=120 ml) after acetone removal (see sorghum bran extracts above).

Ground Whole Sorghum Porridges Cooked with Distilled Water and 70% Aqueous Acetone Sorghum Bran Extracts

Whole sorghum (2.8 g dry basis, starch equivalent) flours were cooked with 25.1 ml distilled water/70% aqueous acetone sorghum bran extracts. Whole white corn porridges cooked with 25.1 ml distilled water/70% aqueous acetone corn bran extract were used as controls.

Corn Starch Porridges Cooked with 70% Aqueous Acetone Sorghum Bran Extracts

Corn starch (3.1 g, as is) and bran extracts were cooked together. Corn starch porridges cooked with distilled water and corn starch porridges cooked with white bran extract were used as controls.

Corn Starch Porridges Cooked with Zein Protein (Sigma Co., St Louis, MO) in Distilled Water and in 70% Aqueous Acetone Sorghum Bran Extracts

Corn starch (3.1 g, as is), zein protein (0.55 g, as is) and 25.1 ml distilled water/bran extracts were cooked together (corn starch: zein ratio of 85:15). Corn starch porridges cooked with distilled water, corn starch porridges cooked with zein in distilled water, and corn starch porridges cooked with zein in white bran extract were used as controls.

Corn Starch Porridges Cooked with Remaining Bran Residues After 70% Aqueous Acetone Extraction in Distilled Water

Corn starch (3. 1 g, as is), bran residue (0.55 g, as is), and 25.1 ml distilled water were cooked together (corn starch: bran residue ratio of 85:15). Double cooked corn starch (with distilled water), double cooked corn starch with white bran residue (with distilled water) were used as controls.

High Amylose Corn Starch Porridges Cooked with 70% Aqueous Acetone Sorghum Bran Extracts

High amylose corn starch (3.1 g, as is) and bran extracts were cooked together. High amylose corn starch porridges cooked with distilled water were used as control.

Porridge Analysis

Assays Used in Preliminary Studies

In vitro Dry Matter Disappearance (IVDMD) Method Used

A quick IVDMD method was only used to conduct preliminary studies to investigate starch digestibility of sorghum porridges. This method was quick and easy to observe differences in digestibility among the porridges. Only hi-tannin, black, and white sorghum varieties were used. Commercial corn starch (Argo) and tannin free white sorghum variety were used for comparisons.

Three ml of bacterial alpha amylase (α-Amylase from *Bacillus* sp. liquid, ≥300 U/g, Sigma-Aldrich) was added directly onto porridges in the canister, and mixture was homogenized using a Polytron homogenizer (Kinematica GmbH, Switzerland) with controlled speed (level 4) for 1 min. Canister was washed into an erlenmeyer with 10 ml 1M Tris maleate buffer (pH=6.9). The erlenmeyer were left in an air oven at 60 ^{0}C for 18 h. Residues were collected by a Whatman filter paper (Grade 41**),** and filter paper was left in the oven at 40 ^{0}C until complete dryness. This analysis was conducted in duplicates for porridges cooked 4 different times.

Assays Used in Main Study

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 starch (dry basis) equivalent wet porridges (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. Fifteen 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, Sigma-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 from 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 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:

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

where C is the percentage of starch hydrolyzed at time t (min), C_{∞} is the equilibrium percentage of starch hydrolyzed after 180 min, and k is the kinetic constant. The variables C_{∞} 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 (EGI)

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:

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

Resistant Starch (RS)

Resistant starch (RS) was determined enzymatically by the method of Goñi et al (1996). Samples from *in vitro* rate of starch digestion were further incubated at 37°C for 13 more hours with constant shaking. The hydrolylate 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.

Microscopy

Selected samples were analyzed by Bright Field Microscopy (Zeiss Universal) using polarized light to observe the structural differences between porridges and their residues after digestion.

Statistical Analyses

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% (α=0.05). The statistical software SPSS v 11.5 (SPSS Inc., Chicago, IL) was used.

CHAPTER IV

SORGHUM GRAIN CHARACTERIZATION AND PRELIMINARY STUDIES

Sorghum grain characters, like other cereals, affect storage, processing, and food quality. Physical grain characteristics such as grain hardness, diameter, color, 1000 kernel weight, density; chemical characteristics such as moisture, protein and starch contents are among the important quality factors of sorghum (Hulse et al 1980, Rooney and Miller 1982).

The objective of this study was to determine the physical and chemical grain characteristics of sorghum varieties used in this study.

Results

Sorghum grain characterization

Moisture and protein contents of all sorghum varieties were similar. Specialty sorghum varieties had significantly ($p<0.05$) lower total starch contents than white sorghum. Black with tannin sorghum had the lowest total starch content compared to other sorghum varieties (Table I).

Thousand kernel weights of the sorghum varieties were significantly ($p<0.05$) different. Kernels of white (2.5 mm, 31 mg), black (2.6 mm, 37.8 mg) and black with tannin (3.0 mm, 35.8 mg) varieties were significantly ($p<0.05$) bigger and heavier than those of hi-tannin (1.8 mm, 23.9 mg) and sumac (1.9 mm, 16.7 mg) (Table I).

TABLE I

Physical and Chemical Characteristics of Sorghum Varieties*

Physical and Chemical Characteristics	White sorghum	Hi-tannin sorghum	Sumac sorghum	Black sorghum	Black-tannin sorghum	LSD
Moisture (%)	11.4a	12.0a	11.9a	11.5a	12.1a	0.8
Protein (% d.b.)	11.7a	11.2a	11.8a	11.7a	11.8a	0.6
Starch (% d.b.)	74.0a	67.5b	68.4b	65.9b	60.4c	2.4
Hardness index (SKHT)	57.7a	50.7c	37.9e	55.6b	42.3d	1.3
Weight (mg)	31.0b	23.9c	16.7d	37.8a	35.8b	0.8
Diameter (mm)	2.5a	1.8b	1.9b	2.6a	3.0c	0.1
TADD(%weight removed)	12.0c	20.2b	20.5b	21.0b	43.8a	1.6
Density (g/cm^3)	1.3a	1.3a	1.3a	1.3a	1.3a	0.0
Thousand kernel weight (g)	30.2b	24.3c	15.8d	44.0a	43.5a	1.3
Test weight (lb/bu)	60.9a	57.5b	59.0a	57.4b	50.5c	1.3
Tannins (mg CE/g d. m.r.)	0.0d	34.4b	42.5a	1.0d	15.0c	1.2
Phenols (mg CE/g d. m.r.)	3.1e	22.5b	28.3a	7.6d	13.8c	1.1
Pericarp	white, thin	red, thin	red, thin	red, thick	red thick	N/A
Pigmented testa	no	yes	yes	no	yes	N/A

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

The hardness indices (HI) of sorghum varieties were significantly ($p<0.05$) different. White sorghum variety had the hardest kernels, with hardness index (HI) of 57.7, sumac sorghum variety with a floury endosperm, was soft, with an HI of 37.9. The softer sorghum varieties had significantly higher percentage of removal in the TADD (Table I). The dissected grains showed a higher proportion of hard endosperm in the white and black sorghum varieties (Fig. 1), and a higher proportion of floury endosperm in the tannin sorghum varieties (hi-tannin, sumac, and black with tannin). The high amount of soft endosperm from the tannin sorghums caused a higher percentage of removal in the TADD and a lower Hardness Index (HI) from the SKHT compared to the white sorghum.

Test weight (bulk density) of white and sumac sorghum varieties were similar; high-tannin and black sorghum were similar, while black with tannin sorghum had significantly ($p<0.05$) lower test weight than the other varieties. All sorghum varieties had similar true densities (Table I).

Specialty sorghum varieties (sumac, hi-tannin, black with tannin and black) had significantly ($p<0.05$) higher total phenol contents than white sorghum. Sumac sorghum had the highest amounts of total phenol contents, followed by hi-tannin, black with tannin, and black sorghum (Table I). Specialty sorghum varieties with pigmented testa (sumac, hi-tannin, and black with tannin) had significant amounts of total phenol and tannin contents.

Fig. 1. Kernels of sorghum varieties: Intact (top) and dissected (bottom). Pictures were taken at 4X magnification.

Whole black sorghum grain had the lowest *L** value (31.4), which means that it was the darkest in color, followed by whole black with tannin sorghum (34.7), and whole tannin sorghum (35.0). White sorghum had the highest *L** value (60.8).

When whole sorghum grains were milled into flours, *L** values of all sorghum varieties were significantly ($p<0.05$) increased (Fig. 2).

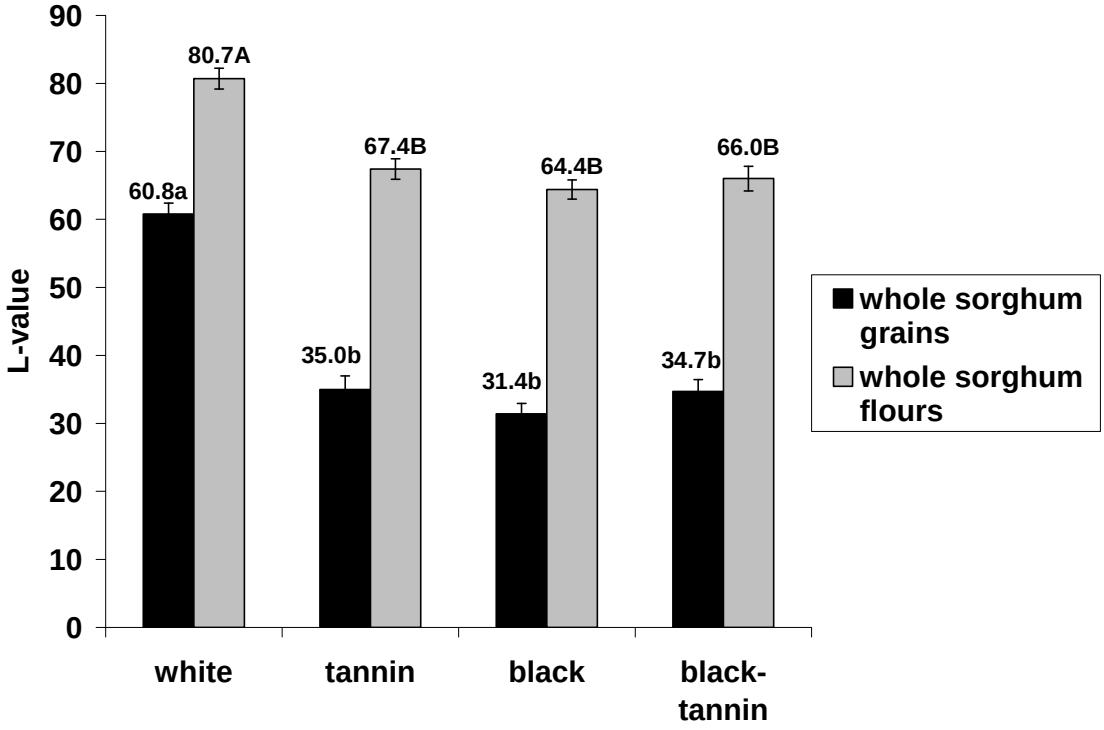

Fig. 2. ***L**** **values of whole sorghum grain and ground whole sorghum grain. Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.**

With the exception of whole white sorghum flour, a* values were positive. Whole tannin sorghum grain had the highest a* value (13.2), which means that it was more red than other sorghum varieties. When whole sorghum grains were milled into flours, *a** values of all sorghum varieties were significantly ($p<0.05$) decreased, because endosperm came out during milling (Fig. 3).

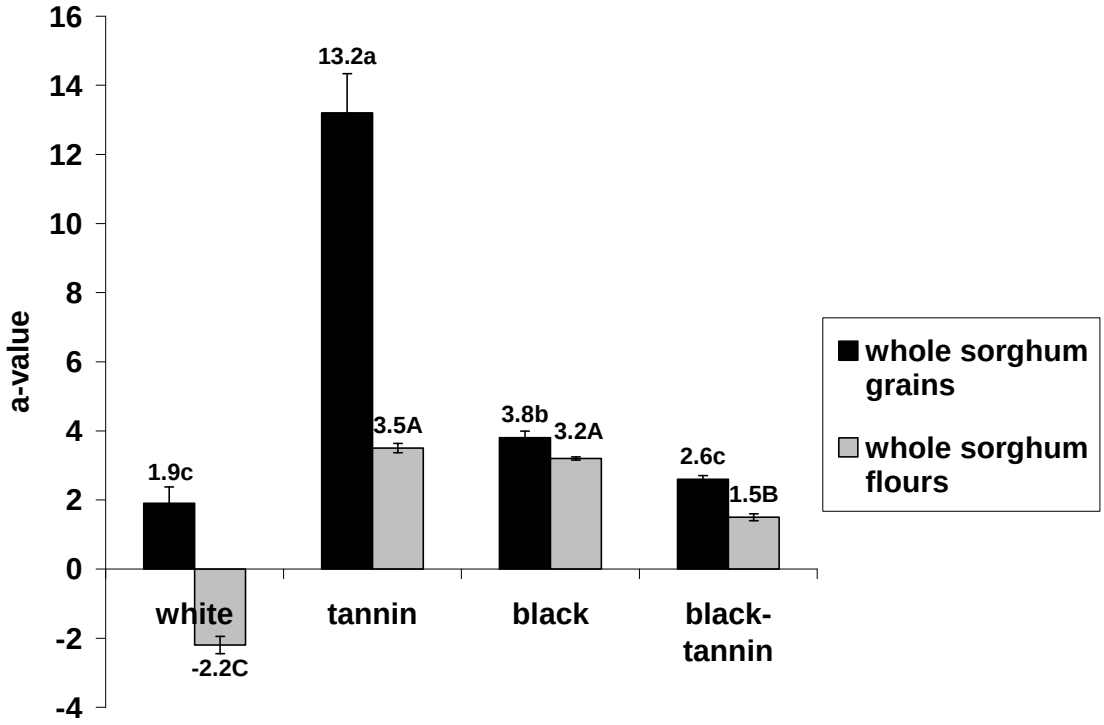

Fig. 3. ***a**** **values of whole sorghum grain and ground whole sorghum grain.**
Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

The *b** values were positive for all samples, which means that they were more yellow than blue. When whole sorghum grains were milled into flours, *b** value of white and tannin sorghum varieties were significantly ($p<0.05$) decreased; black and black with tannin sorghum varieties were significantly ($p<0.05$) increased (Fig. 4).

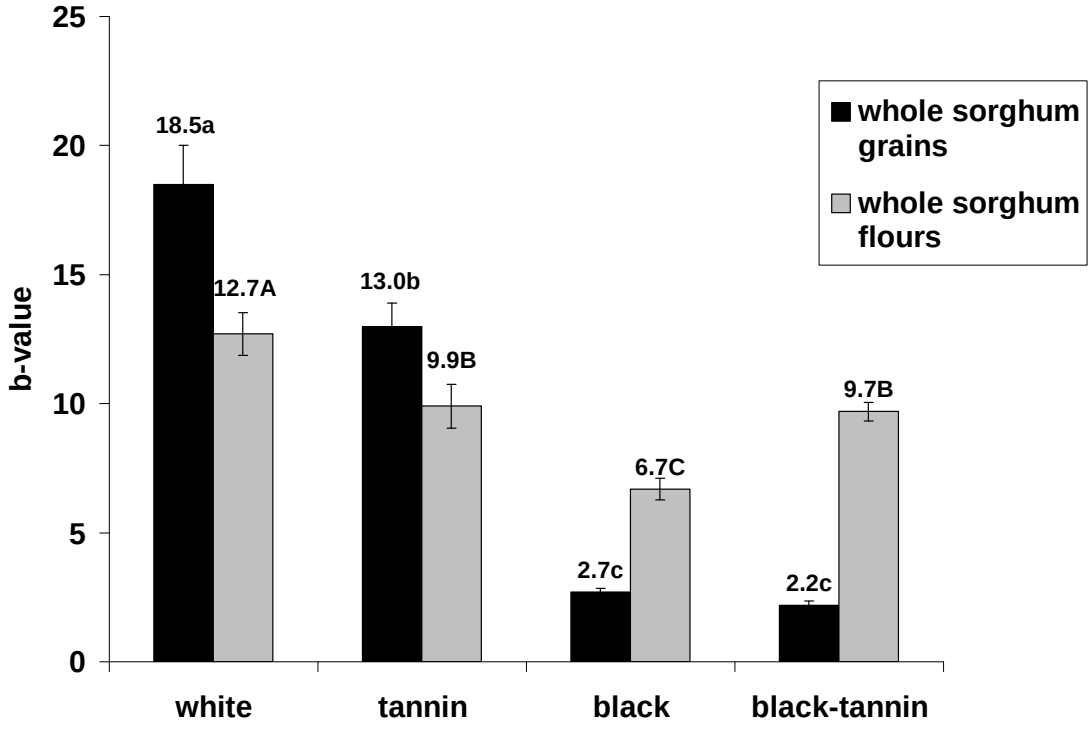

Fig. 4. *b values of whole sorghum grain and ground whole sorghum grain. Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.**

Discussion

All sorghum varieties were morphologically different with significantly ($P<0.05$) different starch contents. White, black, black with tannin sorghum kernels were significantly larger and harder compared to hi-tannin and sumac sorghum kernels (Table I).

Increased starch contents in sorghum varieties gave significantly ($p<0.05$) higher test weights and TADD values. The dissected grains showed a higher proportion of hard endosperm in the white and black sorghum, and a higher proportion of floury endosperm in the tannin sorghum varieties (sumac, hi-tannin, and black with tannin) (Fig. 1). The high amount of soft endosperm in the tannin sorghum varieties caused a higher percentage of removal in the TADD and a lower Hardness Index from the SKHT compared to the white sorghum (Table I). Softer tannin sorghum varieties (sumac, hi-tannin, and black with tannin) had lower decorticated yield (56.2-79.8%) than white sorghum (88 %) variety because the kernels were softer and thus fragmented easily, resulting in endosperm loss with the bran. Sorghum kernels with a high proportion of hard endosperm are suited for de-hulling by pearling procedures (Beta et al 2000), and give higher yield of decorticated product. The starch and protein contents of the sorghum varieties were within the normal ranges reported for sorghum (Rooney and Waniska 2000).

The higher amounts of phenols were in the smaller, softer sorghum kernels (Table I). Sorghum varieties with a pigmented testa and red pericarp color had significantly ($p<0.05$) higher total phenols (13.8-28.3 mg GAE/g) and condensed tannin (15-42.5 mg CE/g) than white sorghum variety (3.1 mg GAE/g, 0 mg CE/g) (Table I).

White sorghum variety had a light pericarp (higher L^* value, Fig. 2) and more yellow color (higher b^* value, Fig. 4), while specialty sorghum varieties had darker pericarp with higher red values. White sorghum variety had the highest L^* values (Fig. 2). This was anticipated, because white sorghum has a white pericarp with a tan secondary plant color.

Whole grains of sorghum varieties had positive *a** values, which means that they were more red than green (Fig. 3). Whole hi-tannin sorghum had a higher *a** value than the other varieties. This was expected since the hilar area, which was covered by the glume during its development, was light red, which affected the redness value. When whole sorghum grains were milled into flours, *a** values of all sorghum varieties were significantly ($p<0.05$) decreased, because milling brings out white endosperm except for black and black with tannin sorghum variety (Fig. 3).

In this study, softer, lighter, smaller sorghum varieties with low thousand kernel weights had significant amounts of total phenols and condensed tannins. These findings provide useful guidelines to understand the relationships between sorghum grain's physical and chemical characteristics with specific emphasis on phenol rich sorghum varieties.

Preliminary Studies

Earlier research by De Castro Palomino Siller (2006) found that tannin sorghum extrudates and porridges were less digestible than that of white sorghum. Condensed tannins seemed to inhibit the starch digestion by limiting starch availability (De Castro Palomino Siller 2006). The aim of the preliminary study was to establish whether further research on the effects of sorghum [*Sorghumbicolor* (L.) Moench] phenolic compounds on starch digestibility of porridges was warranted.

Whole tannin sorghum porridges had significantly ($p<0.05$) higher amounts of residues (after 18 h bacterial alpha amylase digestion) than whole white and black sorghum porridges (Fig. 5). Whole black sorghum porridges had slightly higher amounts of residue than whole white sorghum porridges.

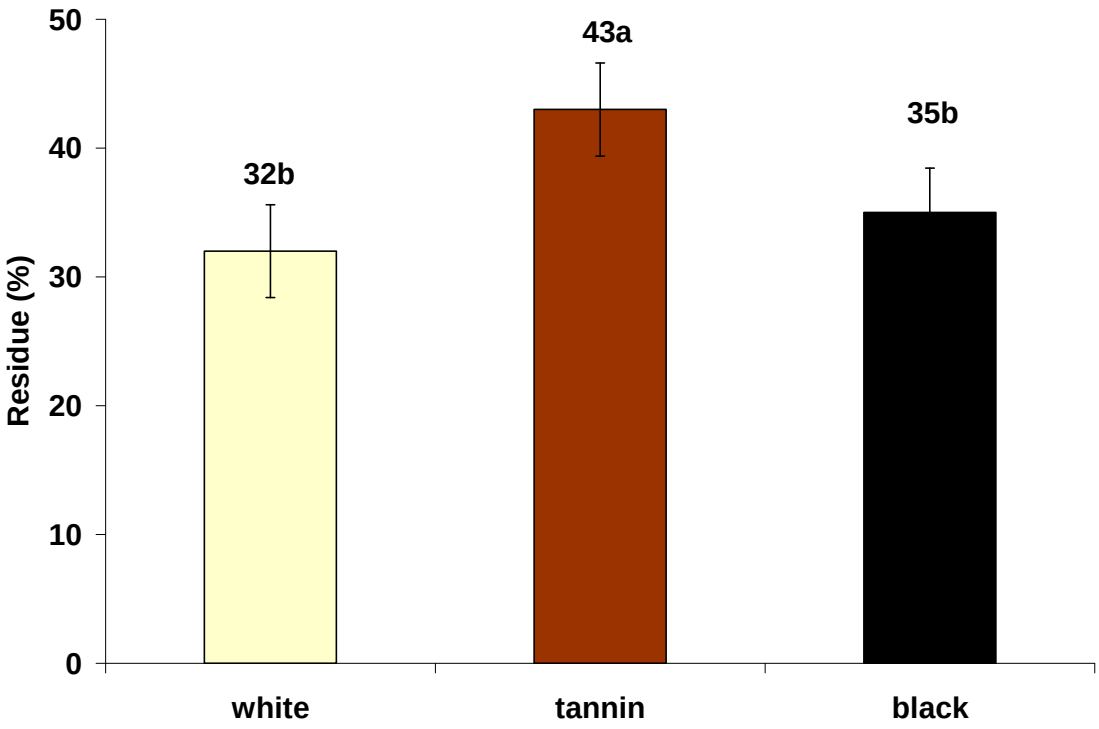

Fig. 5. The residue of whole sorghum grain porridges after 18 h bacterial alpha amylase digestion. Values followed by the same letter are not significantly different ($p<0.05$).

Tannin bran addition to corn starch significantly ($p<0.05$) increased the amounts of residues in the porridges (Fig. 6). Fiber was the main part which was not digested. Tannin bran added to corn starch porridges had significantly ($p<0.05$) higher amounts of residues than residues of non-tannin bran corn starch porridges. The difference between the residue and crude fiber in tannin bran added to corn starch porridges was the resistant starch (2.4%). However, white bran added corn starch porridges had only crude fiber left in the residue. This difference was caused by the presence of phenolic compounds in tannin sorghum brans.

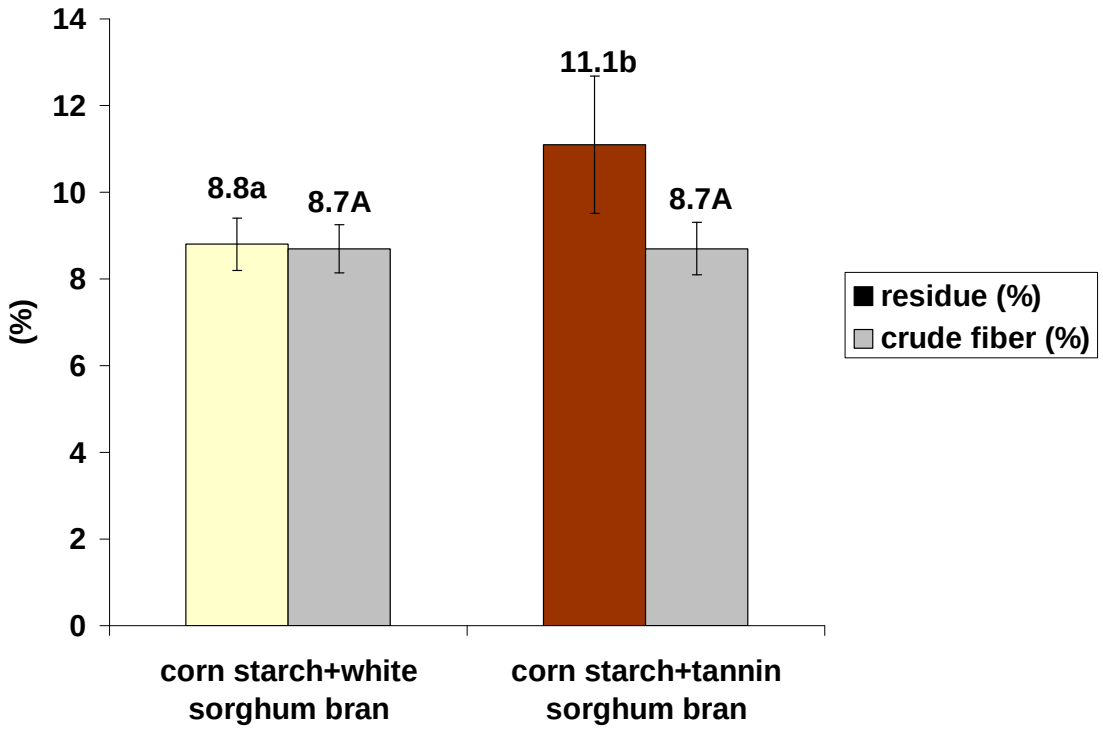

Fig. 6. The residue of corn starch porridges made with tannin and white sorghum brans (corn starch: bran ratio of 85:15) using the IVDMD method. Crude fiber contents were adopted from Gordon (2001). Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

Tannin bran extract addition before or after cooking significantly ($p<0.05$) increased the residue amounts in corn starch porridges (Fig. 7). When tannin extract was added after cooking, the residue was twice as much as residues of corn starch porridges cooked with tannin bran extracts.

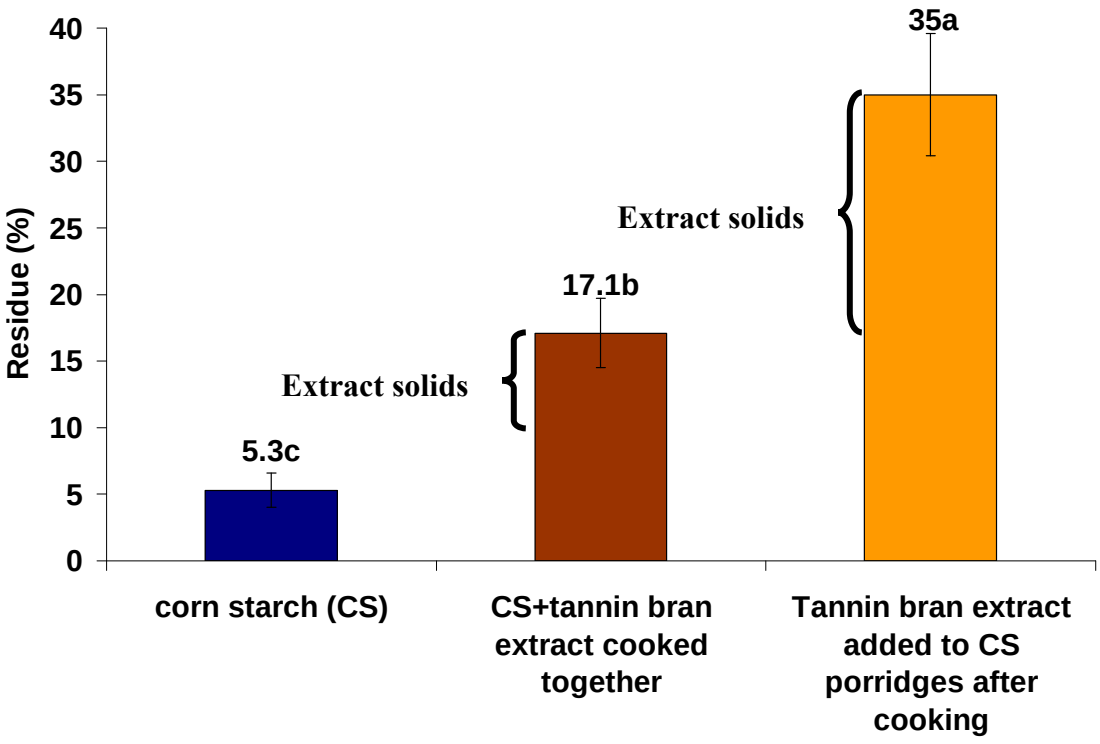

Fig. 7. The effect of tannin bran extract (70% aqueous acetone) addition before or after corn starch was cooked. Values followed by the same letter are not significantly different ($p<0.05$).

This shows that when the tannin bran extract was added to corn starch before cooking, it likely interact with corn starch/protein (less than 1% protein in corn starch) so that they do not inhibit enzymes as much as if they were added after cooking.

The most negatively reported effect of sorghum tannins in animals is reduced feed efficiency (Rooney and Riggs 1971). However, with obesity a major and ever-increasing problem in the developed world (more than 60% of Americans are reported to be overweight) (AHA 2008**)**, this attribute of sorghum tannins/phenols has the potential of helping alleviate the problem. In light of these preliminary studies, the research was conducted in depth and detailed in subsequent chapters.

CHAPTER V

THE EFFECTS OF SORGHUM BRANS ON STARCH DIGESTIBILITY OF SOFT AND HARD SORGHUM ENDOSPERM PORRIDGES

The sorghum brans can be used to fortify bread, cookies and other snacks, to improve the phytonutrient content, as well as dietary fiber and sensory properties.

Kotarski et al (1992), Pedersen et al (2000), Pedersen and Kofoid (2003) found strong relationships between grain hardness index (HI) and *in vitro* dry matter disappearances (IVDMD) of corn and sorghum grains. They reported that differences in starch hydrolysis can partially reflect differences in endosperm structure. Grain hardness also has been reported to be the most important and consistent grain characteristic affecting quality of the porridges (Bello et al 1990, Taylor et al 1996, Rami et al 1998, Aboubacar et al 2002, Rooney et al 1986) and play a major role in the digestibility for both human and livestock (Rooney and Pflugfelder 1986).

This study was designed to investigate the effects of specialty sorghum brans, which are rich in phenolic compounds, on starch digestibility of porridges made with hard and soft endosperm flours. Endosperm porridges cooked with sorghum brans were prepared as described Chapter III, materials and methods.

Results

Total Starch and Phenolic Contents

Specialty sorghum varieties had significantly ($p<0.05$) lower total starch contents than the white sorghum variety (Chapter IV, Table I).

Pure hard and soft sorghum endosperms had similar total starch contents (Fig. 8). Sorghum brans had significantly ($p<0.05$) different total starch contents. White bran had significantly ($p<0.05$) higher total starch content than the other sorghum varieties (Fig. 9).

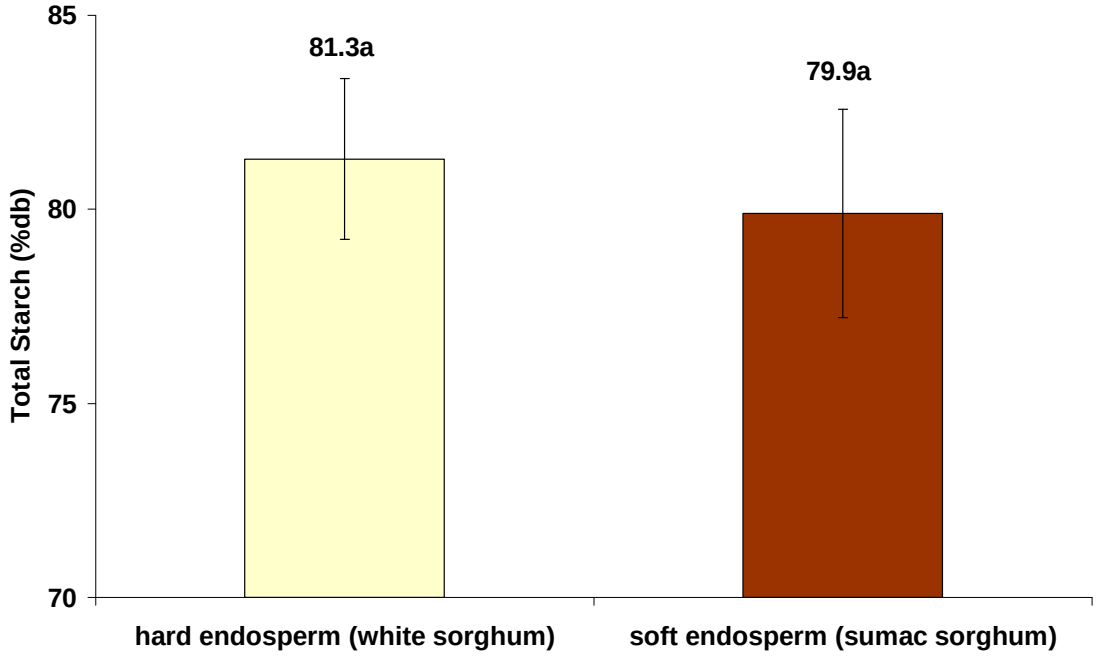

Fig. 8. Total starch contents of pure ground sorghum endosperms. Values followed by the same letter are not significantly different ($p<0.05$).

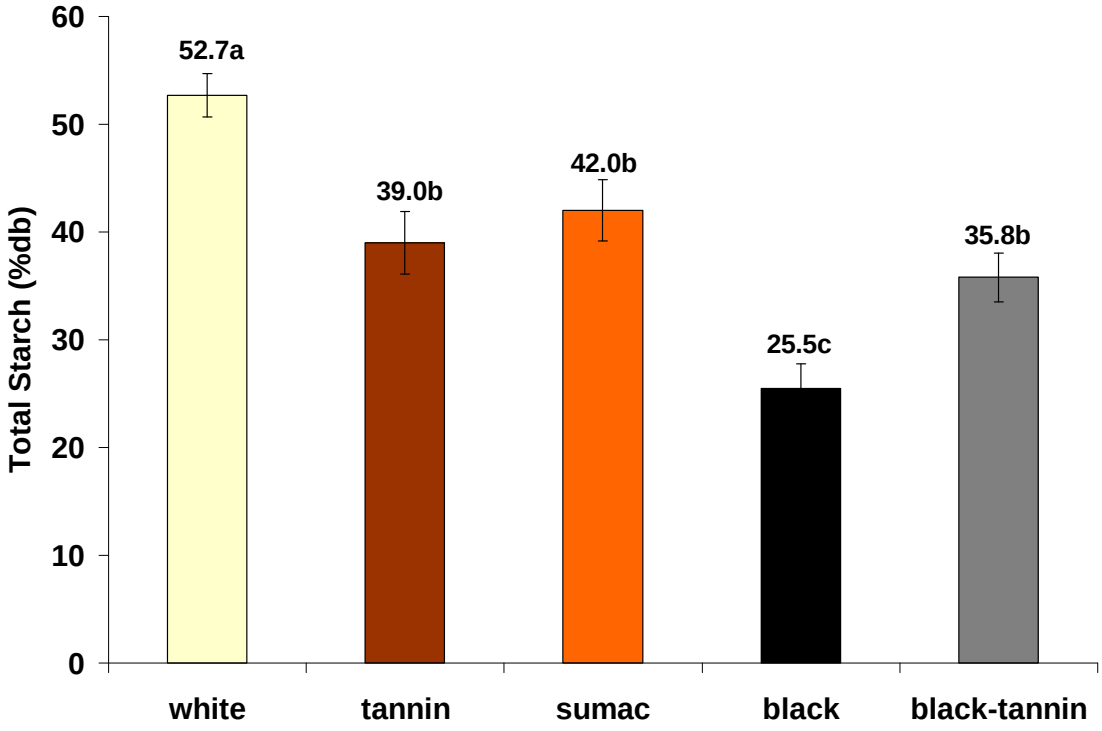

Fig. 9. Total starch content of sorghum brans (10% decorticated). Values followed by the same letter are not significantly different ($p<0.05$).

Whole tannin sorghum grain and its bran had the highest amounts of total phenols and tannin contents, followed by those of black with tannin, and black sorghum varieties. Specialty sorghum brans contained significantly ($p<0.05$) higher amounts of total phenols and condensed tannins than whole sorghum grains (Fig. 10, 11).

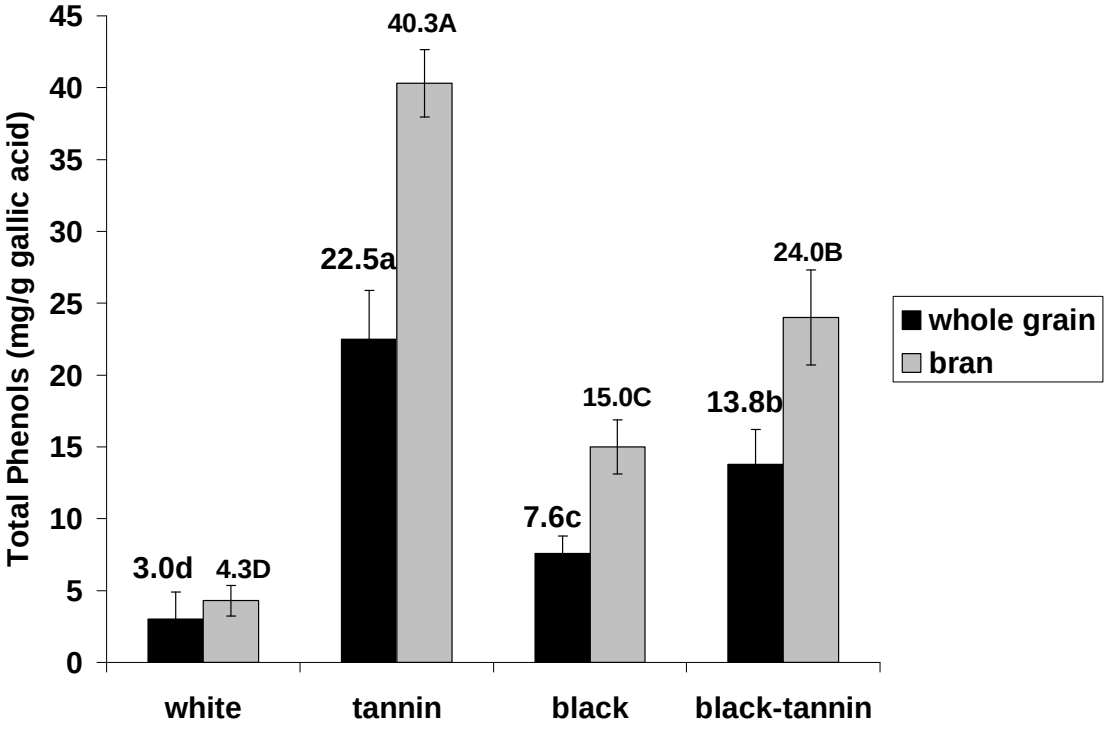

Fig. 10. Total phenol contents (% db) of sorghum brans (10% decorticated) versus ground whole sorghum grains. Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

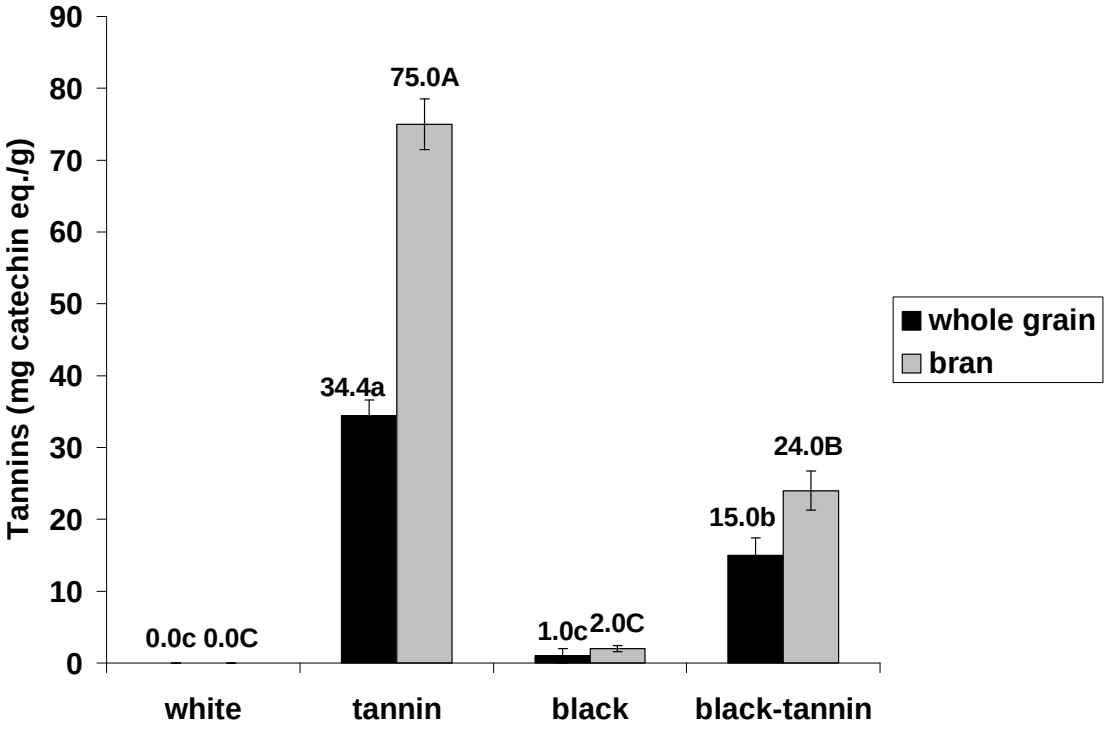

Fig. 11. Tannin contents (% db) of sorghum brans versus ground whole sorghum grains. Values followed by the same uppercase letters are not significantly different (p<0.05), across all samples. Values followed by the same lowercase letters are not significantly different (p<0.05), across all samples.

Black bran particles were larger and sharper than white and tannin brans (Fig. 12). Particle size distributions of sorghum brans were investigated. Black sorghum bran had significantly (P<0.05) larger particles (coarser); while white and black with tannin bran had significantly (P<0.05) smaller particles than the other brans (Table II).

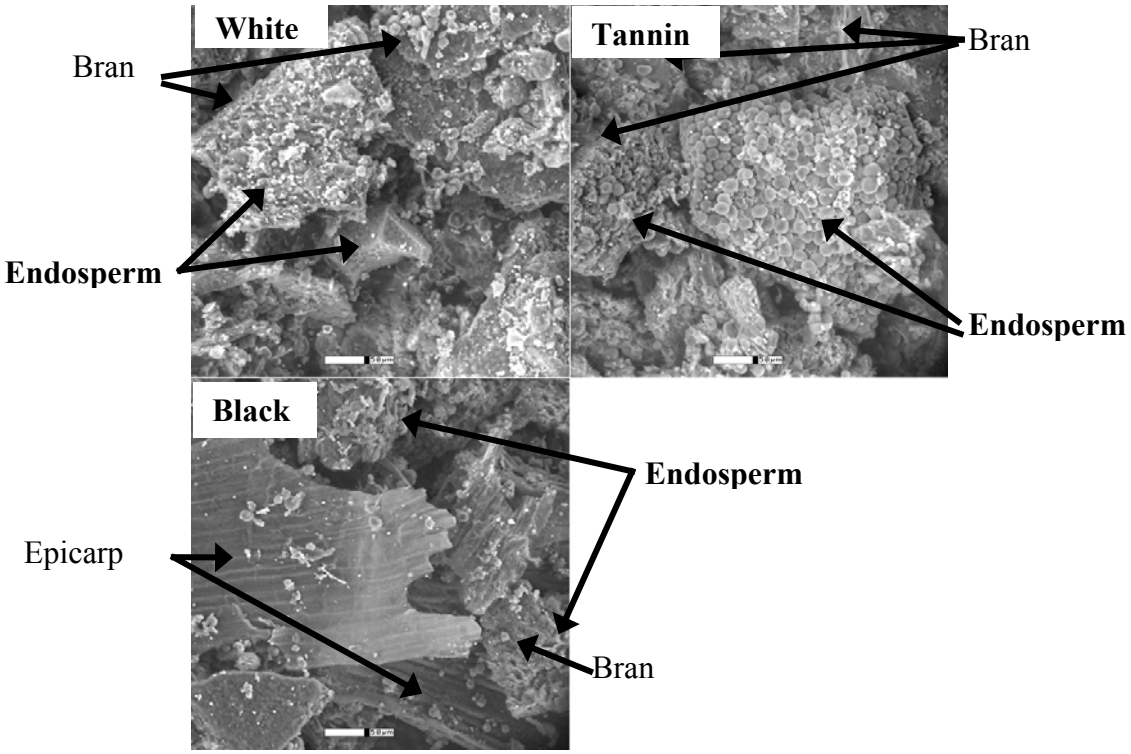

Fig. 12. Structure of selected sorghum brans under Scanning Electron Microscopy (Adopted from Gordon 2001). Pictures were taken at 100X magnification.

TABLE II

Particle Size Distribution (% weight) of Sorghum Brans Used for Porridges*

Sorghum bran type	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 through (<250 µm)
white	0.0a	0.0a	0.9a	4.4a	94.0a	0.3a
high tannin	0.0a	0.0a	1.3a	51.2b	47.5b	0.1a
black	0.0a	2.0a	3.1b	70.9c	23.9c	0.6b
black with tannin	0.0a	1.0a	1.5a	6.2a	91.4a	0.1a
LSD	0.2	0.5	0.6	2.0	2.6	0.2

*Values within the same column with different letters are significantly different at P<0.05.

Starch Digestibility of Ground Soft Sorghum Endosperm Porridges versus Hard Sorghum Endosperm Porridges

Overall soft endosperm porridges had significantly ($p<0.05$) higher starch digestibility than hard endosperm porridges (Fig. 13, Table A-I).

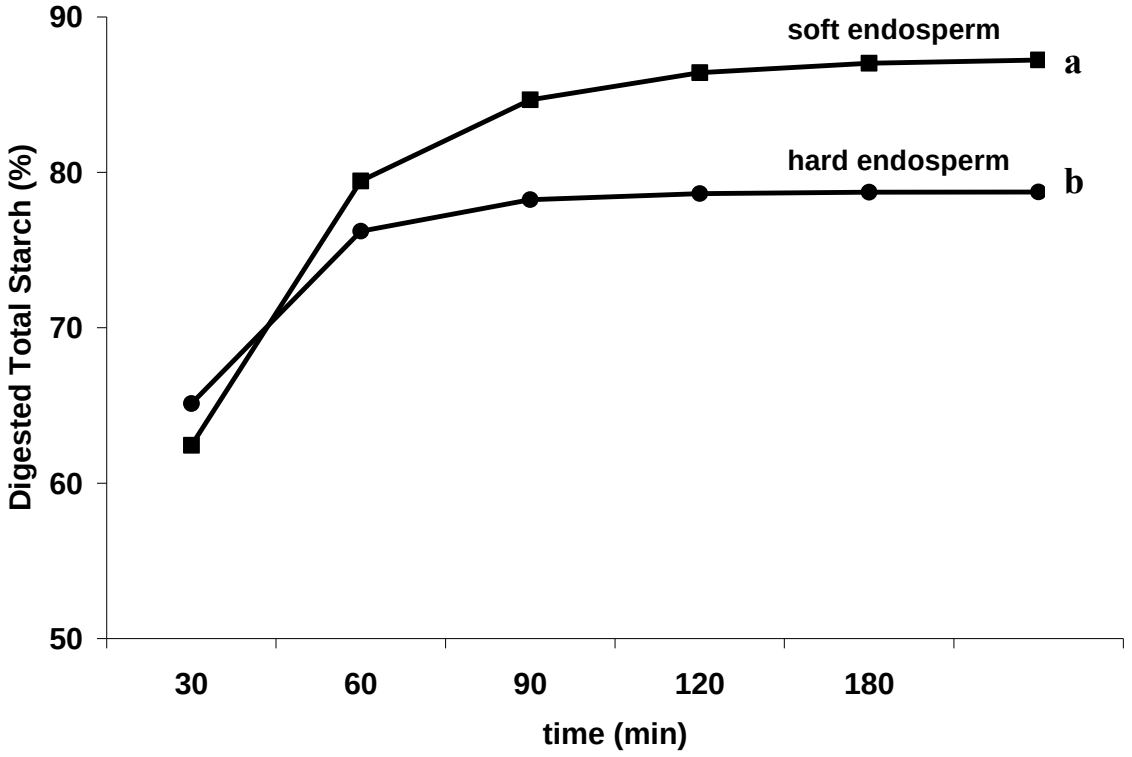

Fig. 13. Starch digestibility of ground hard (white) versus soft (sumac) endosperm porridges. Values followed by the same letter are not significantly different ($p<0.05$).

The Effects of Sorghum Brans on Starch Digestibility of Hard (White) Endosperm Porridges

Black bran significantly ($p<0.05$) increased starch digestibility; tannin and black with tannin bran significantly ($p<0.05$) decreased starch digestibility of hard endosperm porridges (Fig. 14, Table A-II).

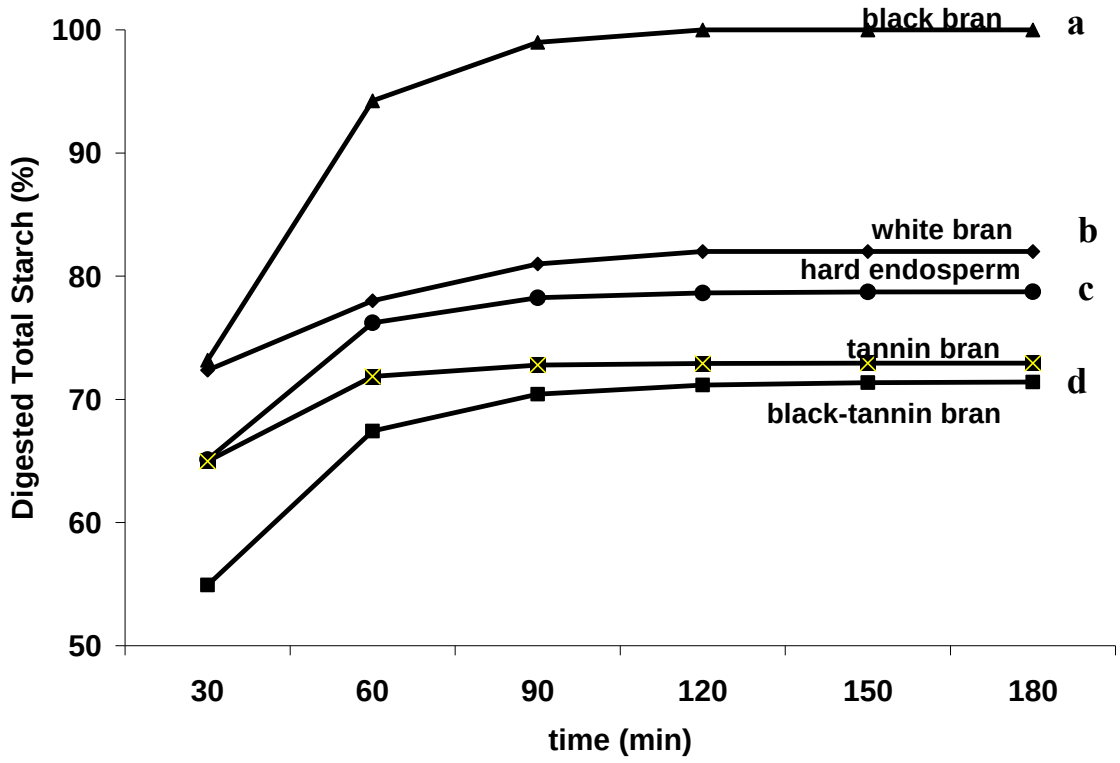

Fig. 14. Starch digestibility of hard (white) sorghum endosperm porridges made with sorghum brans (endosperm: bran ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

The Effect of Sorghum Brans on Starch Digestibility of Soft (Sumac) Endosperm Porridges

Overall, brans of tannin and black with tannin sorghum significantly ($p<0.05$) decreased starch digestibility, while black bran significantly ($p<0.05$) increased starch digestibility of soft endosperm porridges (Fig. 15, Table A-III).

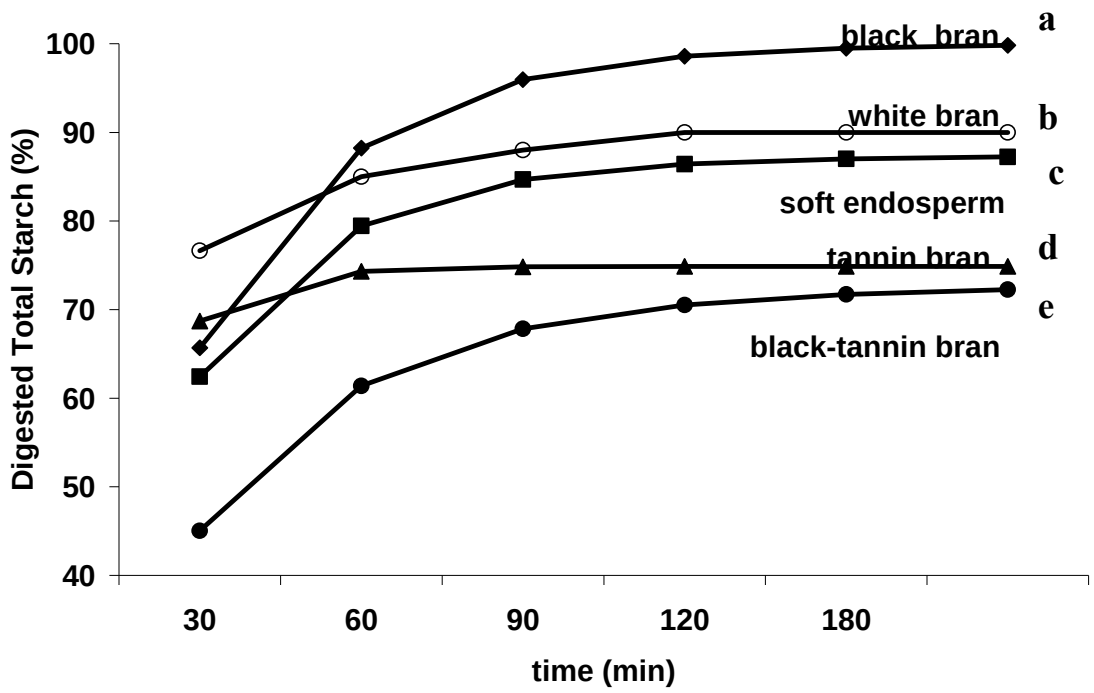

Fig. 15. Starch digestibility of soft (sumac) sorghum endosperm porridges made with sorghum brans (endosperm:bran ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

The Effects of Sorghum Phenols on Starch Digestibility of Hard and Soft Endosperm Porridges

Brans of the same sorghum variety were added to hard and soft endosperm porridges to compare the differences in starch digestibilities between hard and soft endosperm porridges in the presence of sorghum brans. Hard and soft endosperm porridges were used as controls.

Black with tannin sorghum bran addition significantly ($p<0.05$) decreased starch digestibility of hard and soft endosperm porridges. Significant ($p<0.05$) differences existed in digested starch at 30 and 60 min of black with tannin added to endosperm porridges (Fig. 16, Appendix Table A-IV).

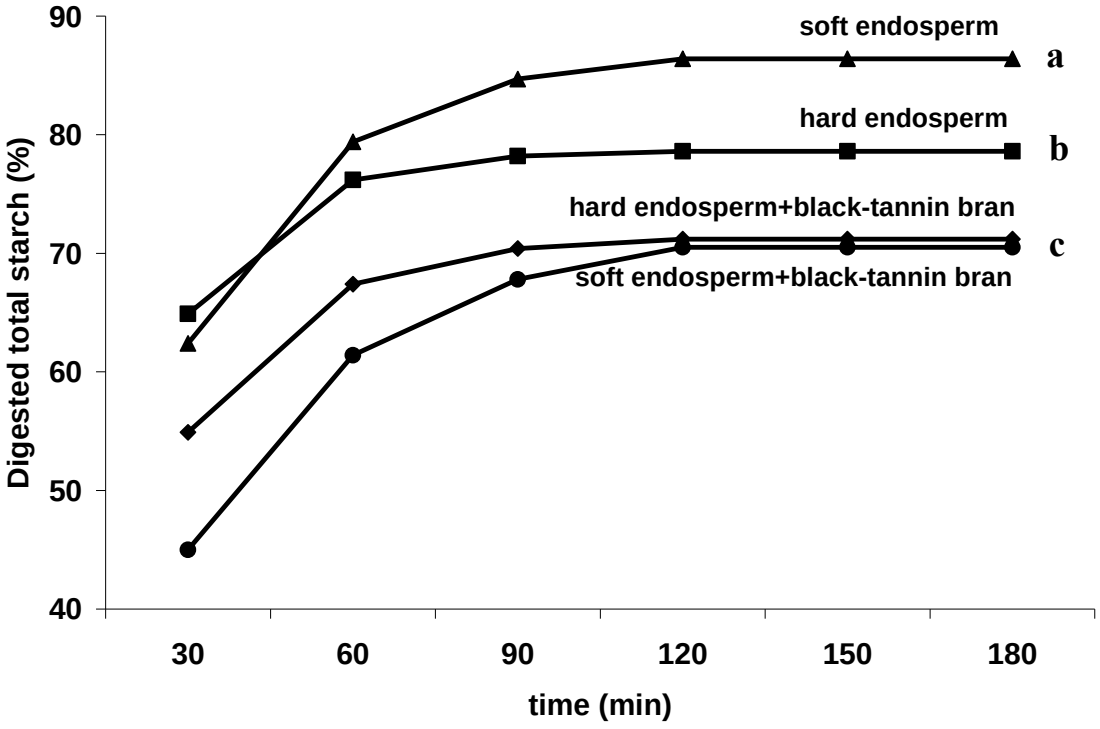

Fig. 16. Starch digestibility of hard (white) versus soft (sumac) endosperm flour porridges in the presence of black with tannin sorghum bran (the ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

Addition of tannin bran to soft and hard endosperm porridges significantly ($p<0.05$) decreased starch digestibility of hard and soft endosperm porridges (Fig. 17, Table A-V).

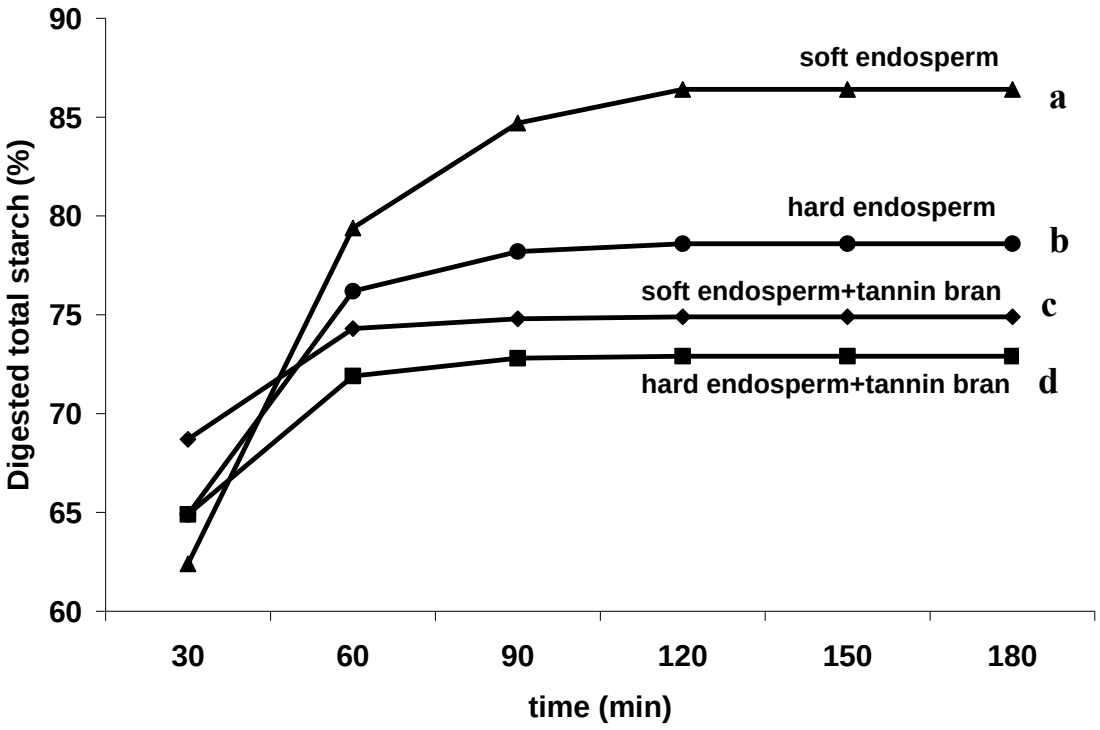

Fig. 17. Starch digestibility of pure hard (white) versus soft (sumac) endosperm flour porridges in the presence of tannin sorghum bran (the ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

Black bran addition to hard and soft endosperm porridges significantly ($p<0.05$) increased starch digestibility (Fig. 18, Table A-VI).

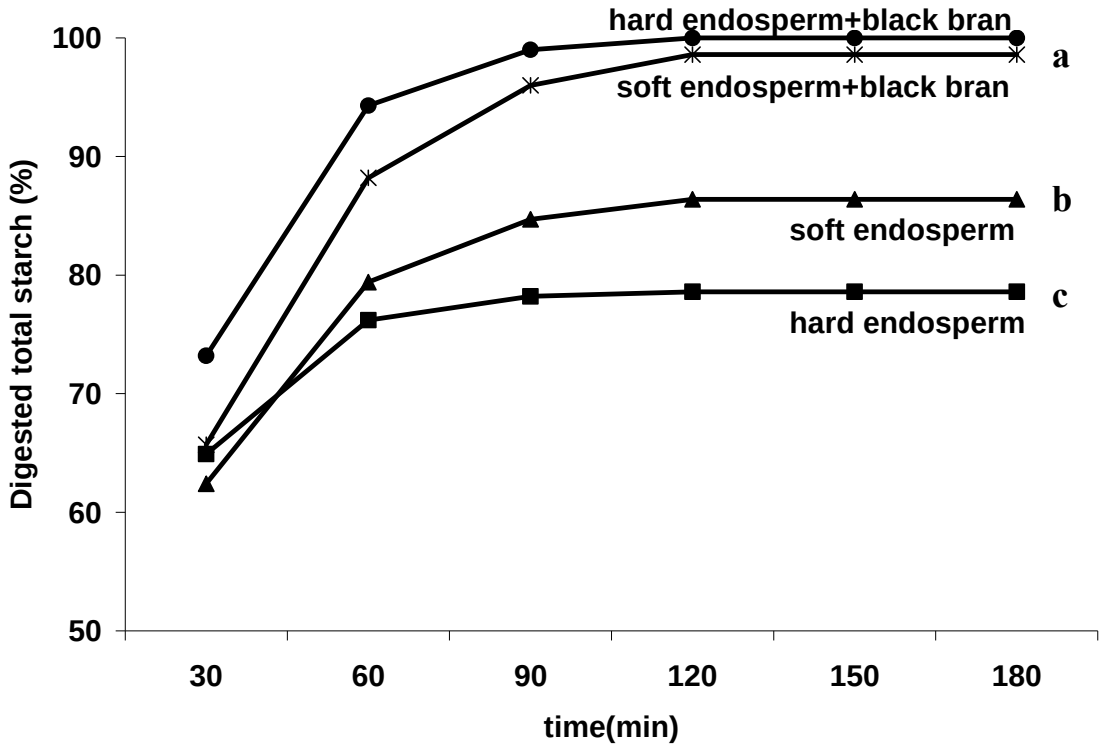

Fig. 18. Starch digestibility of hard (white) versus soft (sumac) endosperm flour porridges in the presence of black sorghum bran. Values followed by the same letter are not significantly different ($p<0.05$).

White sorghum bran added to soft endosperm porridges had significantly ($p<0.05$) higher starch digestibility than white sorghum bran added to hard endosperm porridges (Figure 19, Table A-VII). White sorghum bran addition to hard and soft endosperm porridges significantly ($p<0.05$) increased starch digestibility of the endosperm porridges.

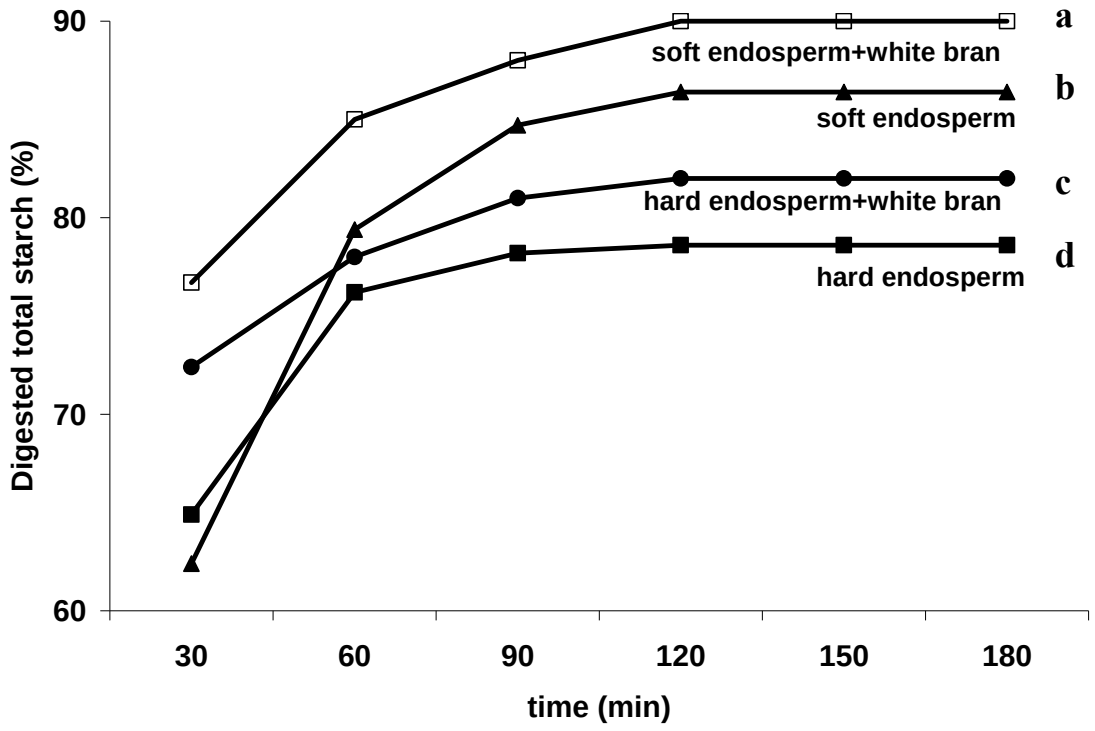

Fig. 19. Starch digestibility of hard (white) and soft (sumac) endosperm porridges in the presence of white bran. Values followed by the same letter are not significantly different ($p<0.05$).

The Effect of Sorghum Brans on EGIs of Hard and Soft Endosperm Porridges

Addition of white and black sorghum bran to porridges significantly ($p<0.05$) increased EGIs of hard and soft endosperm porridges, while tannin and black with tannin bran significantly ($p<0.05$) decreased EGIs of the porridges (Fig. 20).

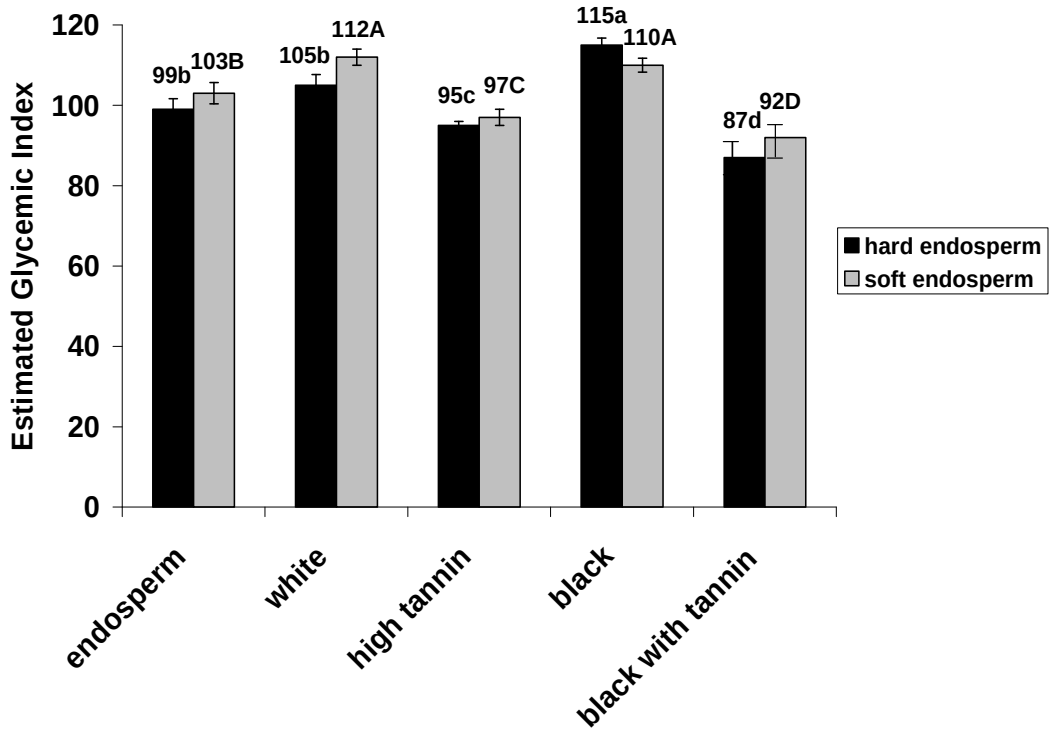

Fig. 20. EGIs of hard (white) versus soft (sumac) sorghum endosperm porridges (endosperm:bran ratio of 85:15). Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

Effect of Sorghum Brans on RS Contents of Soft and Hard Endosperm Porridges

Tannin and black with tannin sorghum brans significantly ($p<0.05$) increased RS contents of both soft and hard endosperm porridges (Fig. 21).

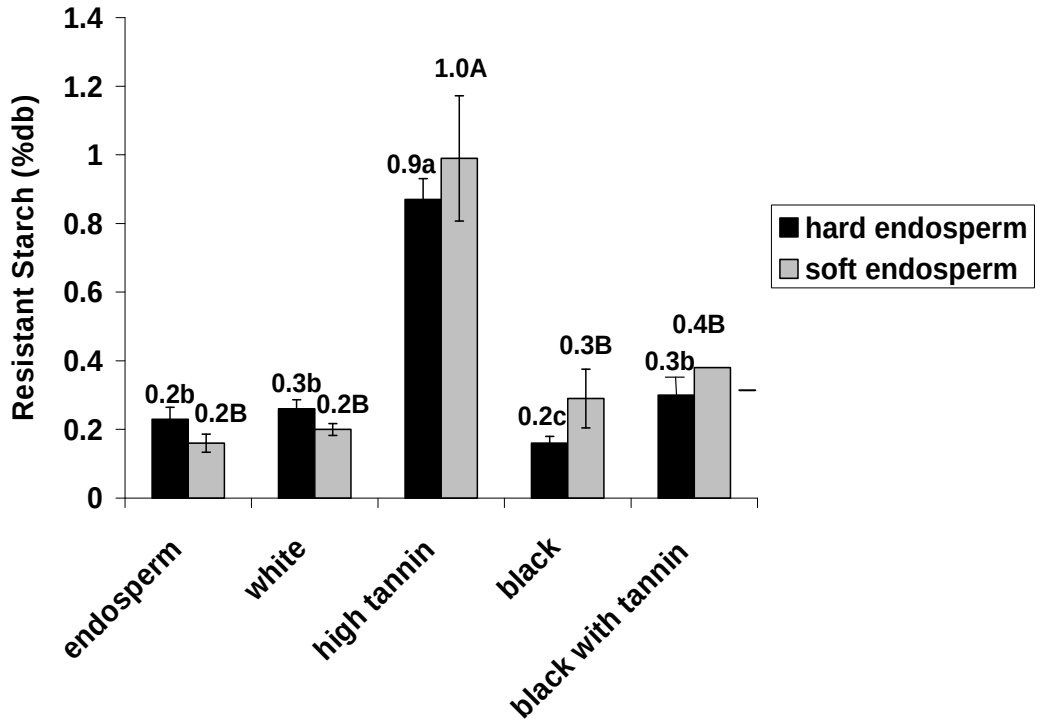

Fig. 21. RS contents of endosperm porridges made with sorghum brans (endosperm: bran ratio of 85:15). Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

Selected Porridges Seen Under Light Microscopy

In this study, phenol rich black bran addition significantly ($p<0.05$) increased starch digestibility and EGIs of the endosperm porridges, while tannin and black with tannin brans significantly ($p<0.05$) decreased EGI of the endosperm porridges. So that porridges made with black bran and the residues remaining after 18 h enzyme digestion were selected for observation under light microscopy. A comparison was made with tannin bran added endosperm porridges and their residues (Fig. 22).

In both porridges, endosperm pieces were glued together. Starch was fully cooked and bran particles were embedded in porridge gel. However, black bran endosperm porridges had more gelatinized starch granules. Black bran endosperm porridges had sharper and bigger bran pieces (Fig. 22a), unlike tannin bran endosperm porridges (Fig. 22b).

Residues of black and tannin bran endosperm porridges had small amounts of gelatinized starch in the bran pieces. Residues of black bran endosperm porridge had agglomerated epicarp pieces (Fig. 22c), which were not observed in residues of tannin bran endosperm porridges (Fig. 22d).

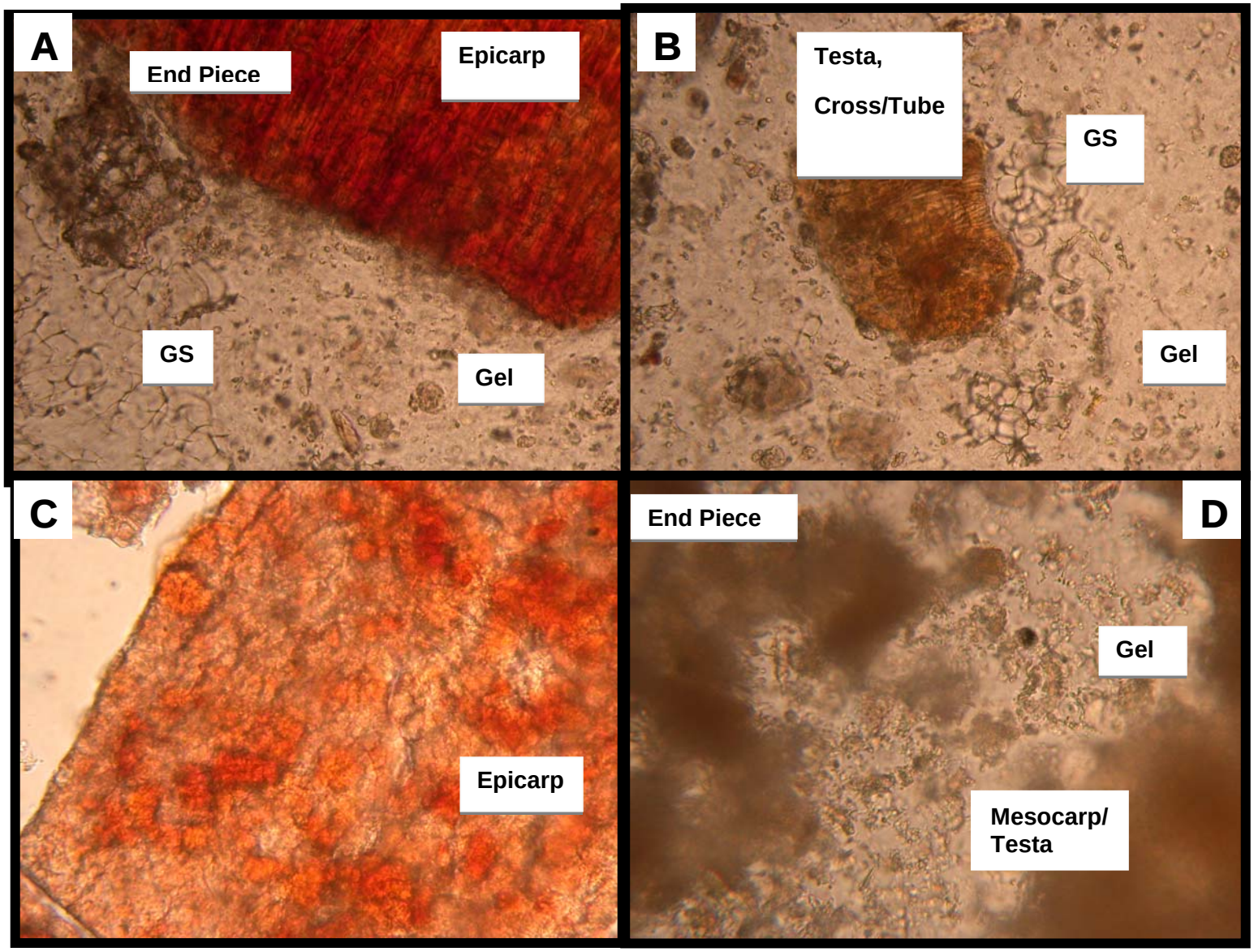

Fig. 22. Selected porridges seen under light microscopy. A: Black bran endosperm porridge, B: Tannin bran endosperm porridge C: Residues (after 18 h enzyme digestion) of black bran endosperm porridge D: Residues (after 18 h enzyme digestion) of tannin bran endosperm porridge, GS: gelatinized starch granules. Picture were taken at 100X magnification.

Discussion

In this study, sorghum varieties with higher amounts of phenolic compounds were softer, and had less total starch contents than those with less phenolic compounds.

Bean and Ioerger (2006) reported that molecular weight, kafirin protein cross linking, and distribution of disulfide bonds and free sulfhydryl bonds differed between hard and soft endosperms. The hard endosperm flours had higher ratio of corneous to floury endosperm than soft endosperm flours. Hard endosperm flour contained abundant protein bodies that surround the starch granule, whereas the soft endosperm was relatively free of protein bodies. Differences in sorghum grain hardness result from adhesion between starch granules and storage proteins. Soft sorghum endosperm has bigger starch granules than hard sorghum endosperm. Smaller granules have a larger surface available for non-covalent bonds with endosperm proteins. Thus, the protein in the hard endosperm (white sorghum) influenced starch gelatinization. As a result, hard endosperm porridges had significantly ($p<0.05$) lower starch digestibility than soft endosperm porridges (Fig. 13, Appendix Table A-I).

Black sorghum bran's larger and sharper particles may have physically disrupted the continuous matrix that holds porridges together, creating weak points, making the porridge more susceptible to enzymes, which resulted in significantly ($p<0.05$) higher starch digestibility, EGI and lower RS contents of endosperm porridges (Fig. 18). Brans of sorghum varieties with condensed tannin (tannin, black with tannin) significantly ($p<0.05$) decreased starch digestibility, EGI and increased RS contents of endosperm porridges, especially soft endosperm porridges. White sorghum bran did not have a significant affect on starch digestibility of endosperm porridges (Fig. 19).

Condensed tannins inhibit enzymes and/or bind prolamin rich protein (kafirin in sorghum) and starch (Asquith and Butler 1986, Davis and Hoseney 1979, Daiber 1975, Beta et al 2000, De Castro Palomino Siller 2006). Since both total starch and starch digestibility assays involve enzyme applications, it is highly probable that tannins complexed with kafirin proteins and starch in soft and hard endosperms, during porridge cooking, which resulted in lower starch digestibilities.

Regardless of endosperm type (hard/soft), presence of phenolic compounds in sorghum brans decreased starch digestibility of endosperm porridges except for black sorghum bran. This study also showed that differences in the structures of sorghum brans affect starch digestibility. *In vivo* studies showed that increased amounts of condensed tannins and total phenols could significantly ($p<005$) lower blood glucose level of diabetic volunteers (Thompson and Yoon et al 1984). Thus the brans of condensed tannins containing sorghum varieties could reduce or slow digestion of sorghum foods and contribute to healthy foods for type II diabetes.

These results suggest that certain specialty sorghum brans, specifically tannin sorghum brans may affect starch digestibility, which would be beneficial for diabetic subjects. The data also suggest that tannin sorghum brans should be consumed by those who want to slow the rate of digestion and improve their health.

CHAPTER VI

EFFECTS OF SORGHUM *SORGHUM BICOLOR* (L.) MOENCH PHENOLS ON RESISTANT STARCH (RS) CONTENTS AND STARCH DIGESTIBILITY OF PORRIDGES

RS intake in the US is much lower than in Europe and Australia (National Starch 2006). Substituting RS for flour in foods is an effective strategy for reducing the glycemic and insulin impact of foods. Moreover, a number of RS-containing ingredients are commercially available today. However, there are no starch products designed with the goal of reducing the rate and extent of starch digestion with increased RS amounts by using food components, such as phenolics.

The objective was to decrease starch digestibility and increase RS in porridges using specialty sorghum phenolics. Corn starch, whole sorghum flours, and hi-maize (high amylose corn) starch was cooked with sorghum bran extracts.

Results

Brans of sorghum varieties were extracted with 70% aqueous acetone to remove extractable phenols. Fig. 23 and 24 show total amounts of phenols and tannins of sorghum brans before and after 70% aqueous acetone extraction. Extraction removed 69-100% (db) of the total phenols from all sorghum brans (Fig. 25); 92.4-96.6% (db) of tannins from tannin sorghum brans (Fig. 26).

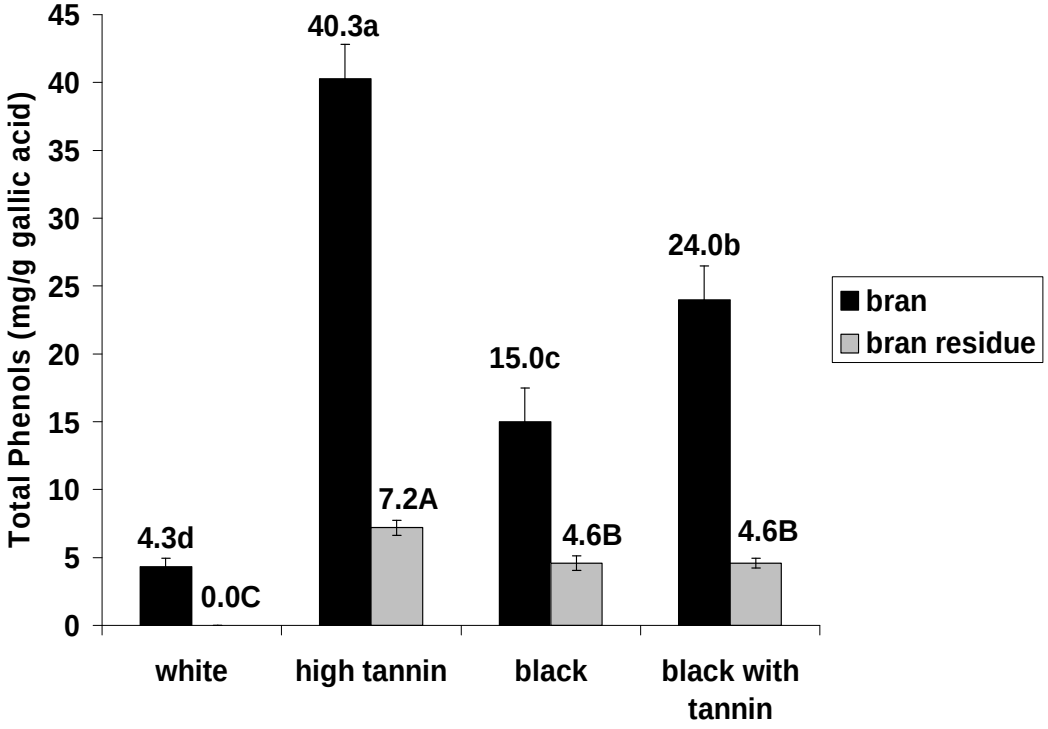

Fig. 23. Total phenol contents (% db) of sorghum brans (bran) before and after extraction with 70% aqueous acetone (bran residue). Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

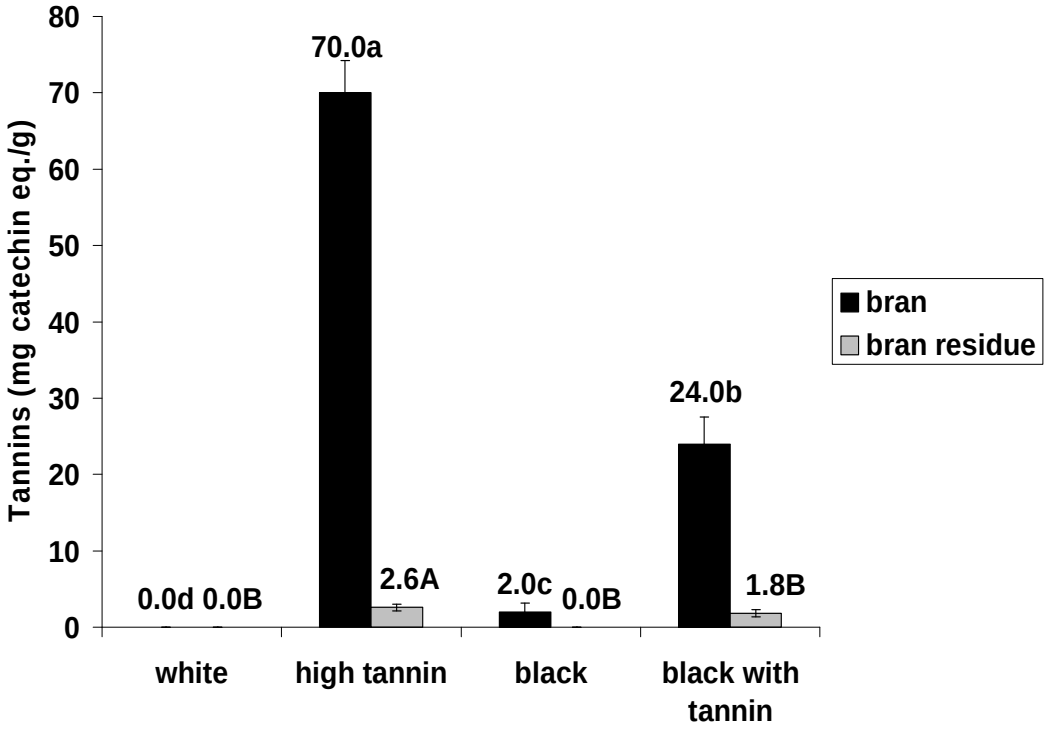

Fig. 24. Tannin contents (% db) of sorghum brans (bran) before and after extraction with 70% aqueous acetone (bran residue). Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

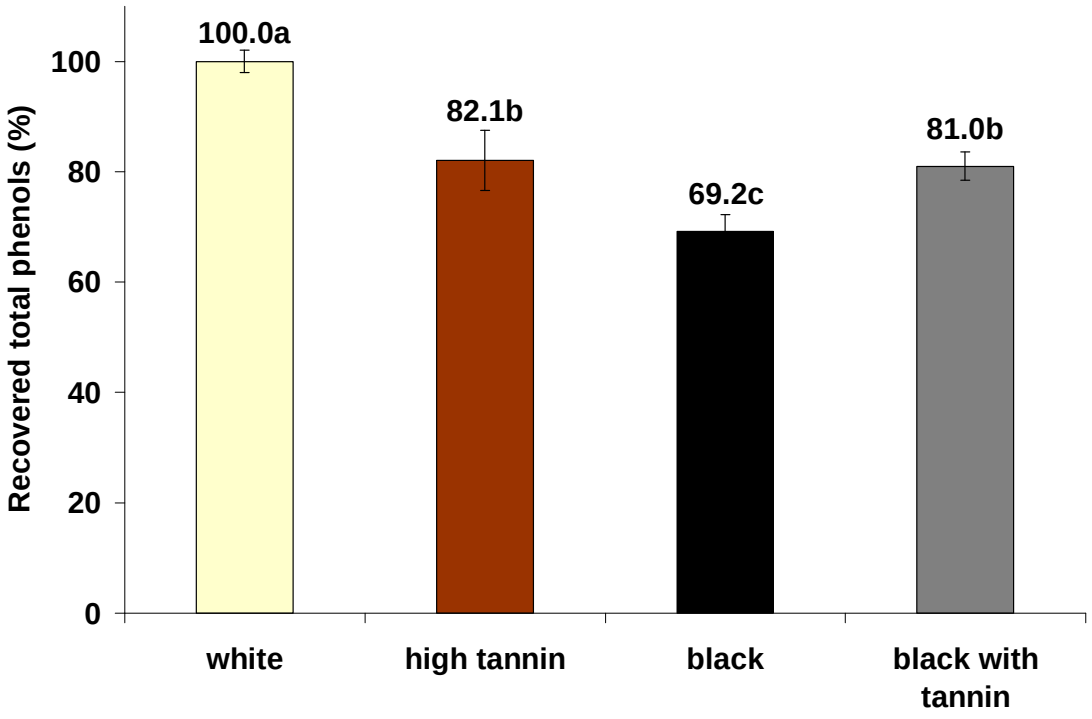

Fig. 25. Recovery (%db) of total phenols with 70% aqueous acetone extraction of sorghum brans. Values followed by the same letter are not significantly different ($p<0.05$).

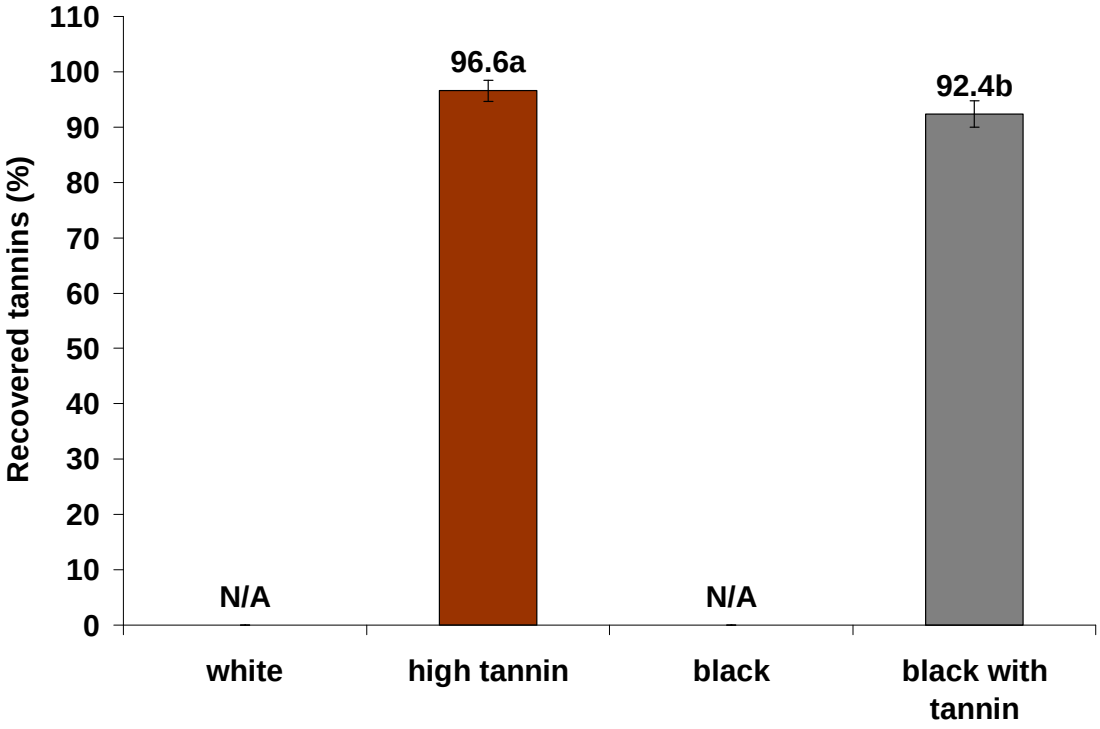

Fig. 26. Recovery (%db) of tannins extracted with 70% aqueous acetone from sorghum brans. Values followed by the same letter are not significantly different ($p<0.05$).

Sorghum bran extracts in 70% aqueous acetone are shown in Fig. 27. Tannin bran extract had significantly ($p<0.05$) higher solid contents than the other extracts, while black and black with tannin extracts had similar solid contents (Fig. 28).

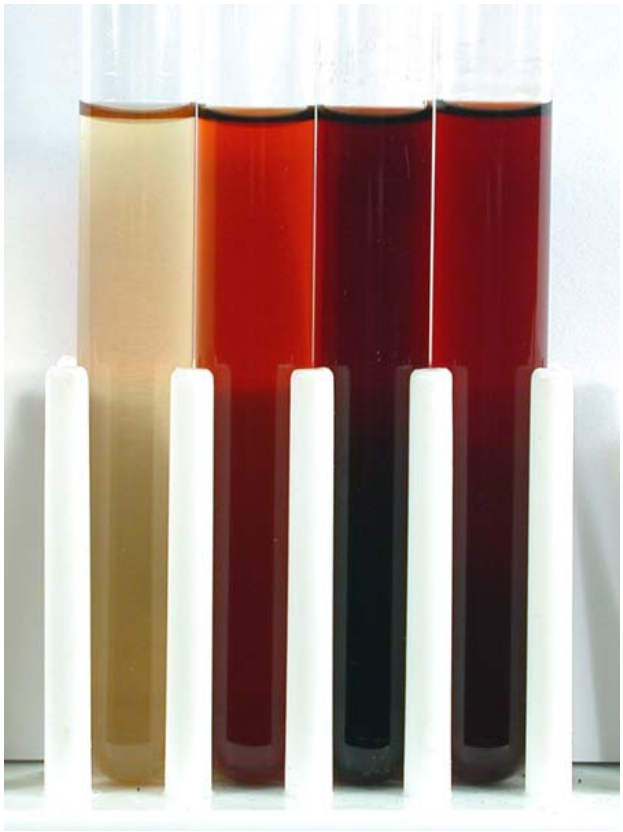

Fig. 27. Sorghum bran extracts in 70% aqueous acetone. From left to right, white, tannin, black, black with tannin sorghum bran extracts, respectively.

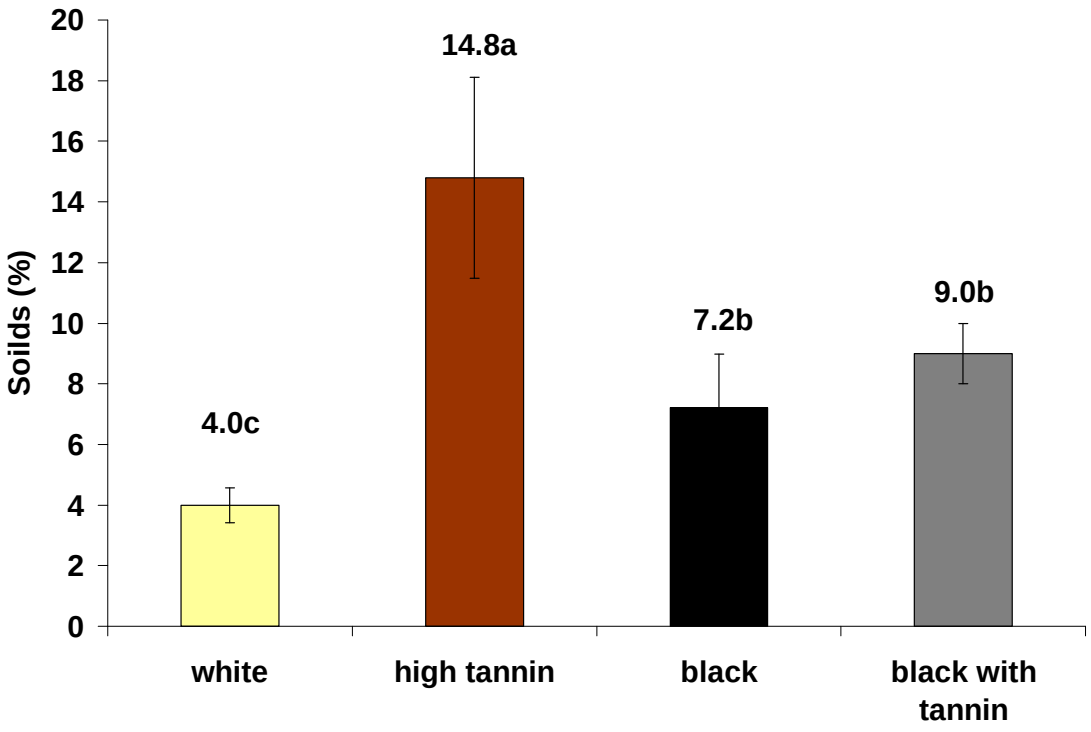

Fig. 28. Solid contents (%) of 70% aqueous acetone sorghum bran extracts after removal of acetone. Values followed by the same letter are not significantly different ($p<0.05$).

In order to observe if the solvent used to extract phenolics from sorghum brans affected the starch structure, sorghum brans were observed under polarized light with bright light microscopy before and after 70% aqueous acetone extraction. Starch granules were intact with maltese crosses and bran fiber was undamaged (Figure 29).

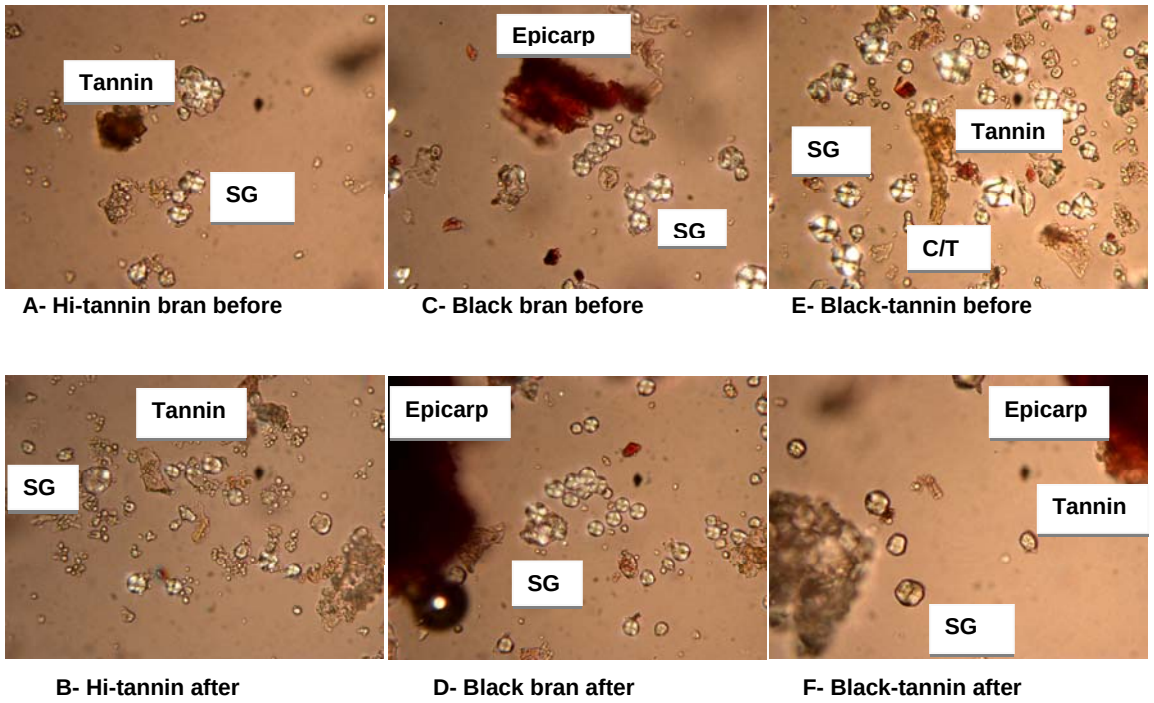

Fig. 29. Selected sorghum brans observed under polarized light microscopy before and after 70% aqueous acetone extraction. SG: starch granules, C/T: cross and tube cells. Pictures were taken at 250X magnification.

The Starch Digestibility of Corn Starch Porridges Cooked with Sorghum Bran Extracts Obtained by Different Solvents

Distilled water, absolute methanol, 70% aqueous acetone, and 70% aqueous ethanol were used to remove sorghum phenols (Chapter III, Materials and Methods). Extracts were cooked with corn starch (3.1 g, dry basis) in a rapid visco-analyzer (RVA).

Digestibility of corn starch porridges made with white bran extracts was similar among the solvents (Fig. 30).

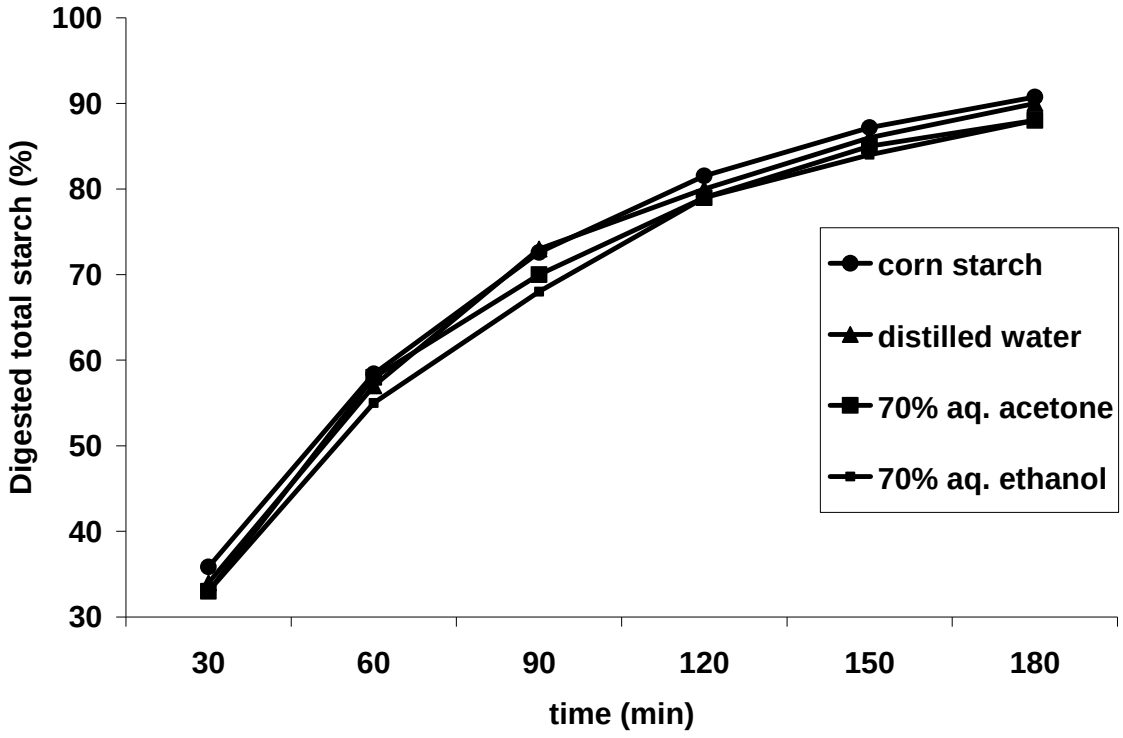

Fig. 30. Starch digestibility (%db) of corn starch porridges cooked with white sorghum bran extracts obtained with different solvents.

Aqueous acetone is commonly used for tannin extractions in fruits and sorghums (Garcia-Viguera et al 1998, Awika and Rooney 2004). The lowest starch digestibility of corn starch porridges was obtained by 70% aqueous acetone extractions (Fig. 31).

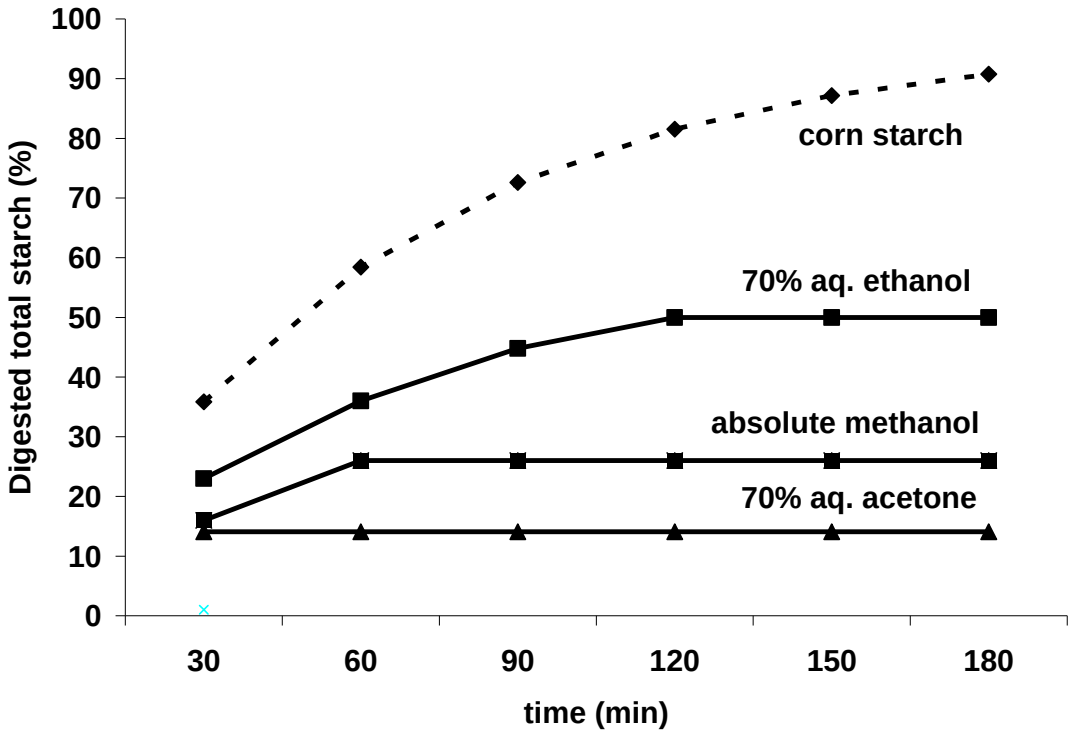

Fig. 31. Starch digestibility (%db) of corn starch porridges cooked with tannin sorghum bran extracts obtained with different solvents.

Starch digestibility of corn starch porridges containing black bran extracts (Fig. 32) had differences among the solvents.

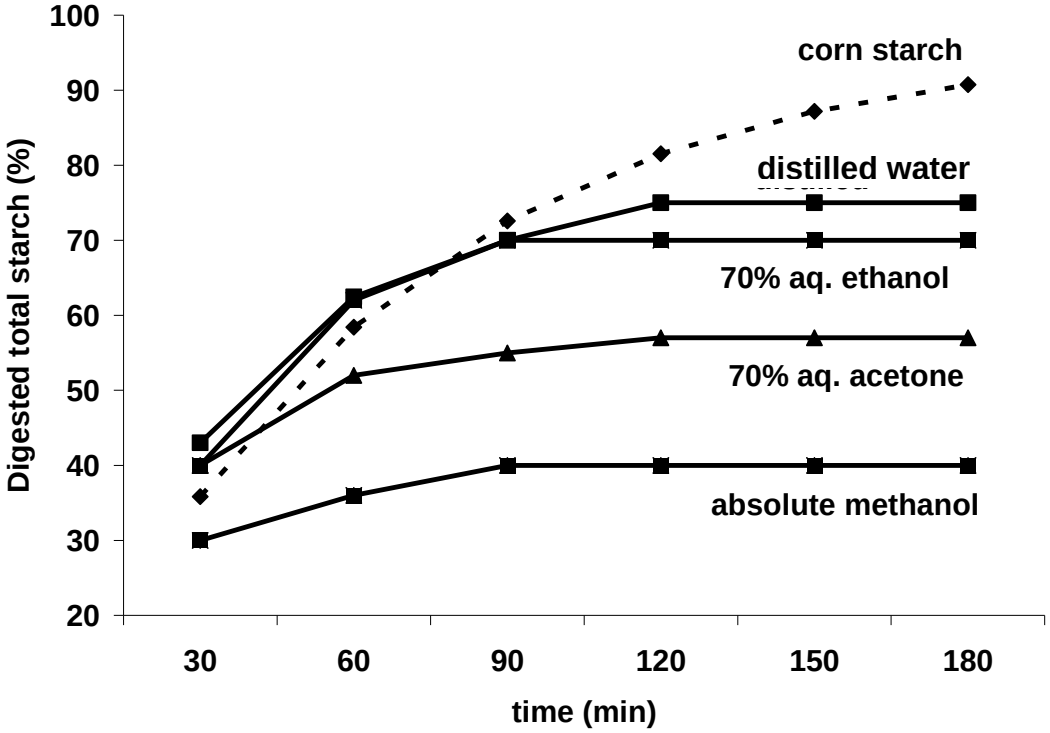

Fig. 32. Starch digestibility (%db) of corn starch porridges cooked with black sorghum bran extracts obtained with different solvents.

Starch digestibility of corn starch porridges made with black-tannin bran extracts (Fig. 33) had differences among the solvents.

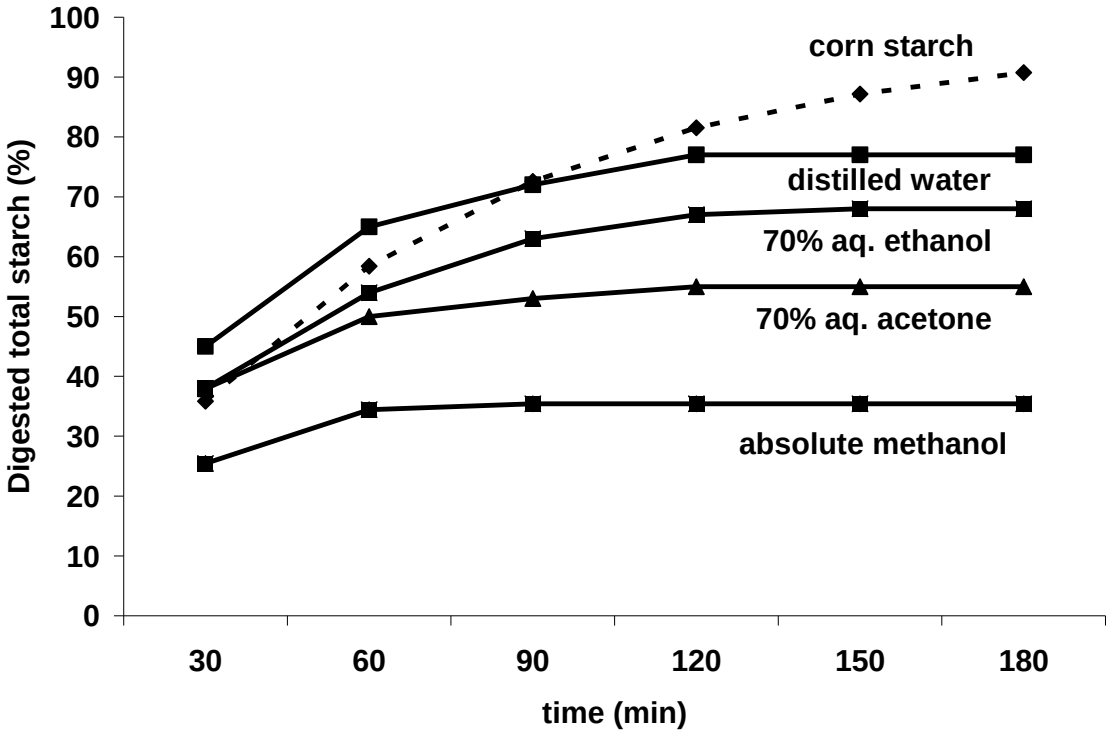

Fig. 33. Starch digestibility (%db) of corn starch porridges cooked with black with tannin sorghum bran extracts obtained with different solvents.

Seventy percent (70%) aqueous acetone for tannin bran, and absolute methanol for black and black with tannin brans were the best solvents, because the most reduction in starch digestibility of corn starch porridges were obtained with those solvents for each type of sorghum brans. However, 70% aqueous acetone was used for phenol extractions of sorghum brans for the remaining investigations to be consistent. The water remaining after acetone removal did not affect enzyme hydrolysis of corn starch porridges (data not shown).

The Effects of Double Cooking-cooling Cycles on Digestibility of Corn Starch Porridges

Some studies (Skrabanja and Kreft, 1998, Berry 1986; Bjorck and others 1990, Mann and Toeller 2001) showed that multi cooking-cooling cycles could modify gelatinization processes, increase retrogradation, and consequently increase levels of resistant starch (RS3). In our study, corn starch went through double cooking-cooling cycles with varying amounts of water (Chapter III, Materials and Methods, Appendix Table C-I) to investigate if starch digestion could be modified. Single cooked corn starch porridges were used as controls.

Double cooking with limited water at first cooking significantly ($p<0.05$) decreased starch digestibility, EGI, and increased RS contents compared to single cooking (Fig. 34, 35, 36, Table B-I). Therefore, all porridges were made with double cooking with limited water at first cooking, and called double cooked corn starch porridges (Chapter III, Materials and Methods).

The Effects of 70% Aqueous Acetone Sorghum Bran Extracts on Starch Digestibility of Double Cooked Ground Whole Sorghum Grain Porridges

Ground whole sorghums were cooked with distilled water (Fig. 37) and 70% aqueous acetone sorghum bran extracts. Ground whole sorghum porridges had significantly ($p<0.05$) lower starch digestibility than whole grain corn porridges. Whole specialty sorghum porridges cooked with distilled water had significantly ($p<0.05$) lower starch digestibility than whole white sorghum porridges cooked with distilled water (Fig. 38, Table B-II).

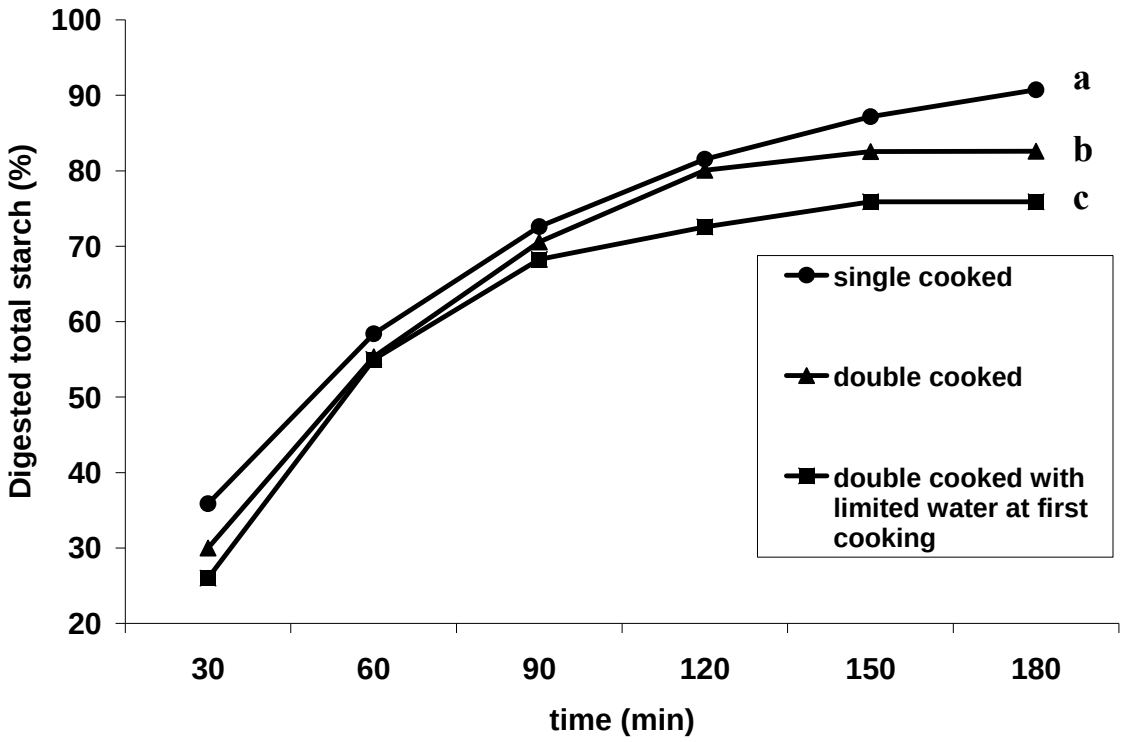

Fig. 34. Starch digestibility (%db) of double cooked corn starch porridges. Values followed by the same letter are not significantly different ($p<0.05$).

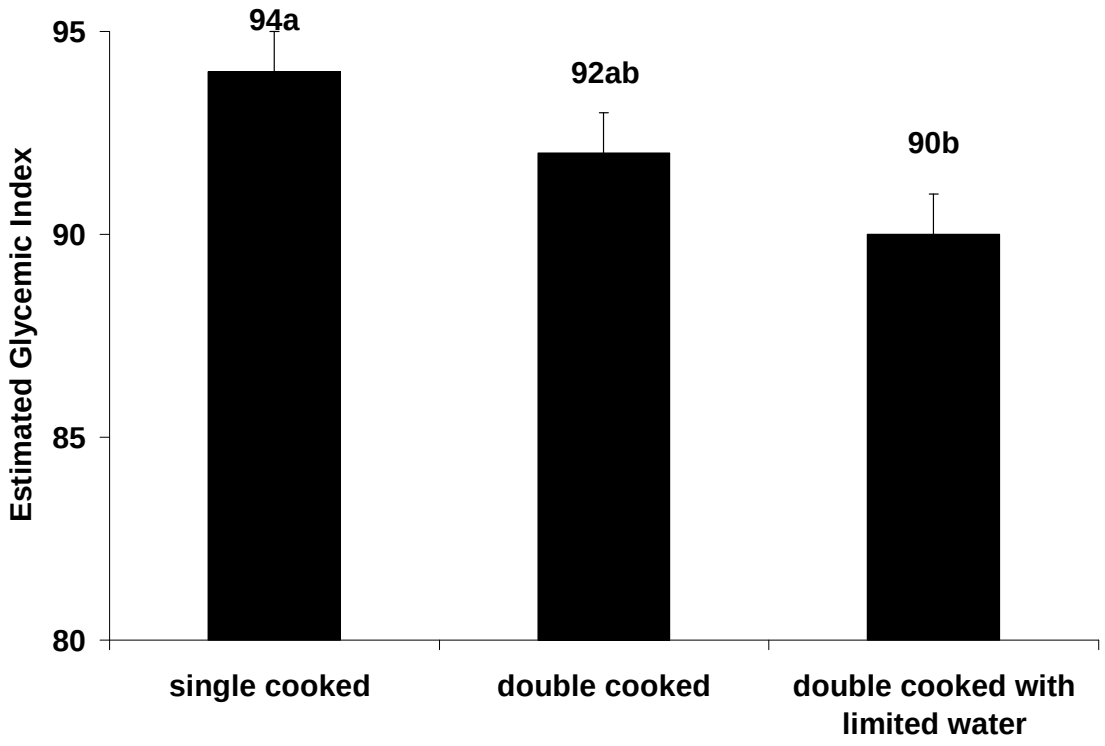

Fig. 35. EGIs of double cooked corn starch porridges. Values followed by the same letter are not significantly different ($p<0.05$).

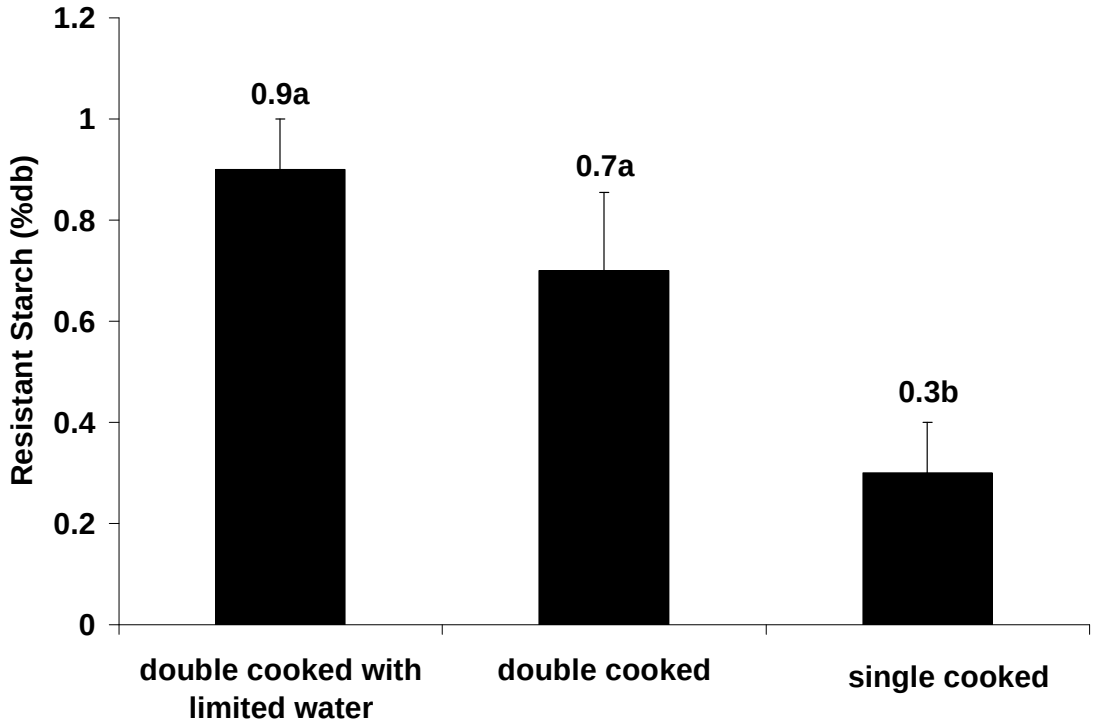

Fig. 36. RS (%db) of double cooked corn starch porridges. Values followed by the same letter are not significantly different ($p<0.05$).

Fig. 37. Double cooked ground whole corn and sorghum porridges cooked with distilled water.

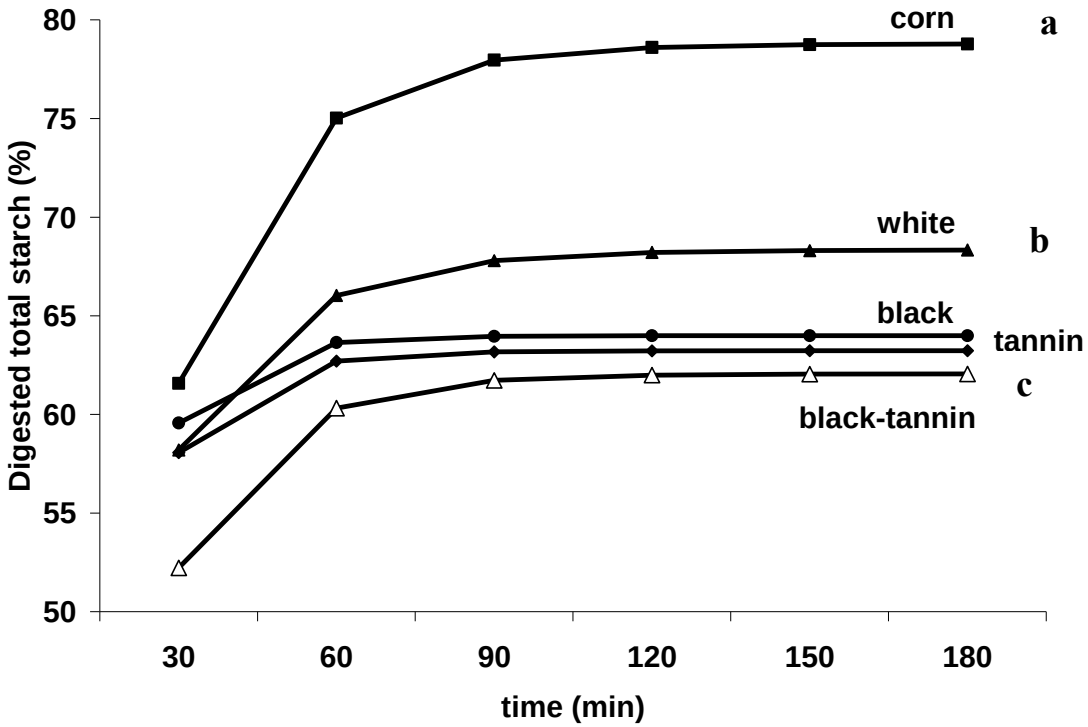

Fig. 38. Starch digestibility (%db) of double cooked ground whole sorghum and corn porridges made with distilled water. Values followed by the same letter are not significantly different ($p<0.05$).

Ground whole sorghum porridges cooked with sorghum bran extracts showed significantly lower starch digestibility compared to ground whole corn porridges cooked with 70% aqueous acetone corn bran extracts (Fig. 39, Appendix Table B-III). Cooking ground whole sorghums with their corresponding bran extracts significantly ($p<0.05$) decreased starch digestibility of the porridges (except whole white sorghum) compared to those cooked with distilled water (Table B-III).

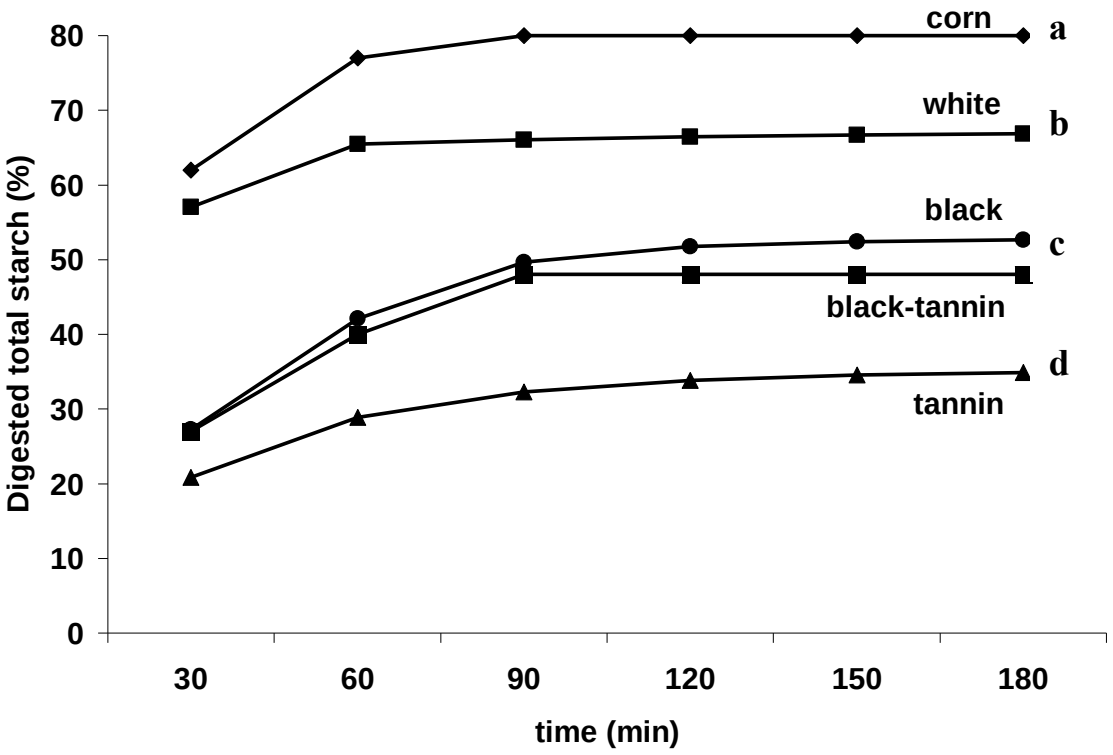

Fig. 39. Starch digestibility (%db) of double cooked ground whole sorghum grain porridges made with 70% aqueous acetone sorghum bran extracts. Values followed by the same letter are not significantly different ($p<0.05$).

Whole sorghum porridges cooked with distilled water had significantly ($p<0.05$) lower EGIs than those of whole corn porridges (Fig. 40). Only black with tannin sorghum porridges cooked with distilled water had significantly ($p<0.05$) lower EGIs than whole white sorghum porridges cooked with distilled water (Fig. 40).

Whole sorghum porridges cooked with their bran extracts had significantly ($p<0.05$) lower EGIs than whole corn porridges cooked with corn bran extract. Cooking whole specialty sorghum varieties with their corresponding bran extracts significantly ($p<0.05$) decreased EGIs compared to whole white sorghum porridges cooked with white sorghum bran extracts. Whole tannin porridges cooked with its bran extracts had the lowest EGIs, followed by whole black with tannin and black sorghum porridges (Fig. 40).

Addition of specialty sorghum bran extracts to their whole grain porridges significantly ($p<0.05$) decreased EGI values compared to the porridges cooked with distilled water (Fig. 40).

Whole tannin, black with tannin and black sorghum porridges cooked with distilled water had significantly ($p<0.05$) higher RS content than white sorghum porridges cooked with distilled water (Fig. 41).

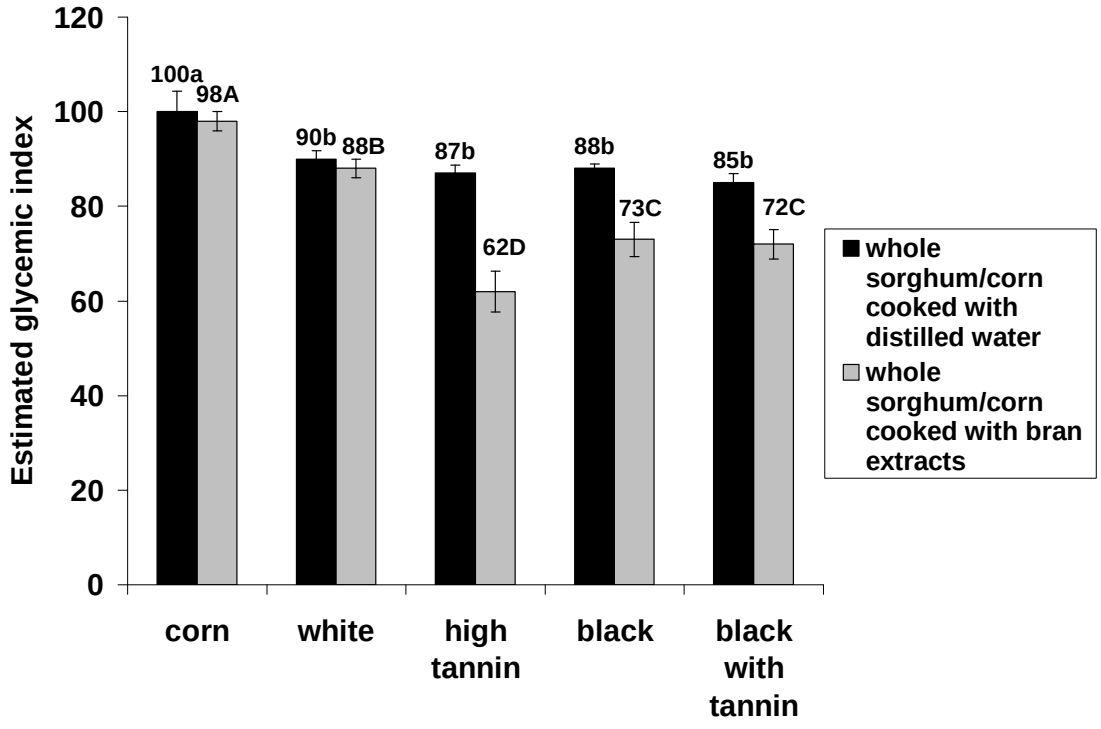

Fig. 40. EGIs of double cooked ground whole sorghum grain porridges made with distilled water versus 70% aqueous acetone sorghum bran extracts. Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

Cooking whole specialty sorghum with their bran extracts significantly ($p<0.05$) increased RS content by 2-7 times compared to their porridges cooked with distilled water.

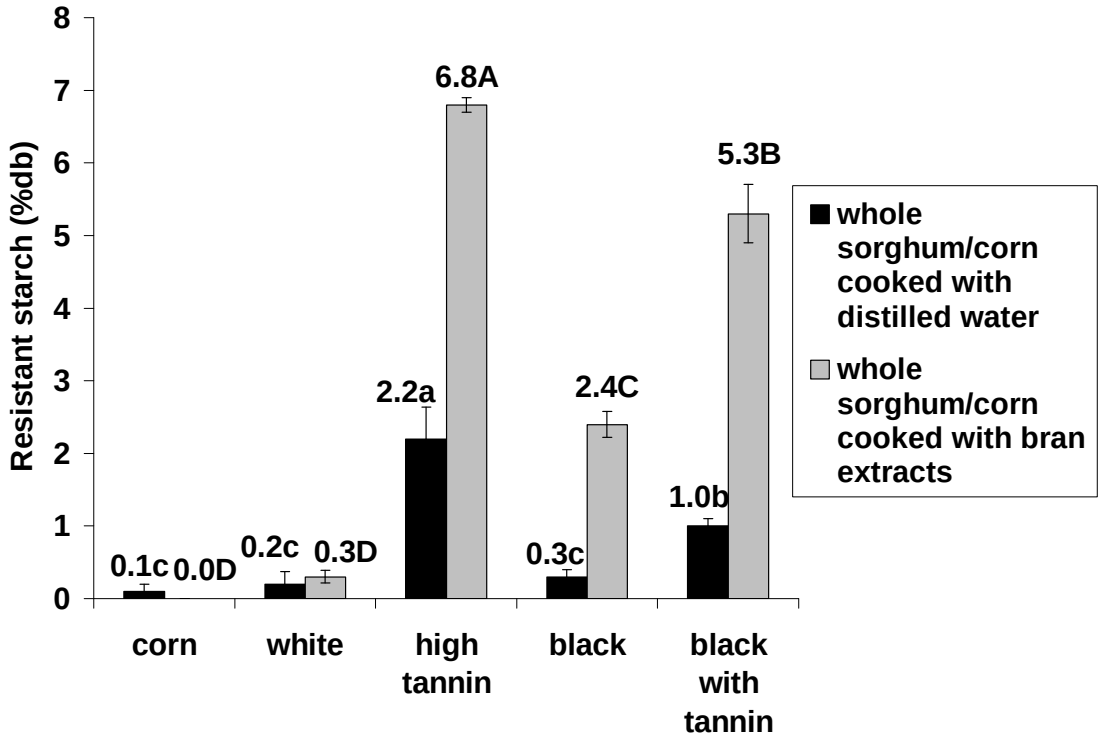

Fig. 41. RS (%db) contents of double cooked ground whole sorghum grain porridges made with distilled water versus 70% aqueous acetone sorghum bran extracts. Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

Whole tannin and black with tannin sorghum porridges cooked with their bran extracts had significantly ($p<0.05$) higher RS values than whole black sorghum porridges cooked with black bran extracts (Fig. 41).

The Effects of Zein Protein on Starch Digestibility of Double Cooked Corn Starch Porridges Made with 70% Aqueous Acetone Sorghum Bran Extracts

Corn (zein) and sorghum (kafirin) prolamins were the least digestible proteins. However, low protein digestibility of tannin sorghum was attributed to the presence of tannins that bind protein and reduced digestibility (Chibber et al 1980). On the other hand, other studies showed that tannin free sorghum varieties still showed lower protein digestibility compared to other cereals (Axtell et al 1981, Hamaker et al 1986, Rom et al 1992). So corn starch, zein protein, and sorghum bran extracts were used to make porridges to investigate the effects of zein protein and phenolic compounds on digestibility of corn starch porridges.

Zein protein significantly ($p<0.05$) decreased the digestibility of corn starch porridges. However, corn starch porridges cooked with zein protein and white bran extracts had significantly ($p<0.05$) lower starch digestibility than corn starch porridges made with zein and specialty sorghum bran extracts (Fig. 42, Table B-IV).

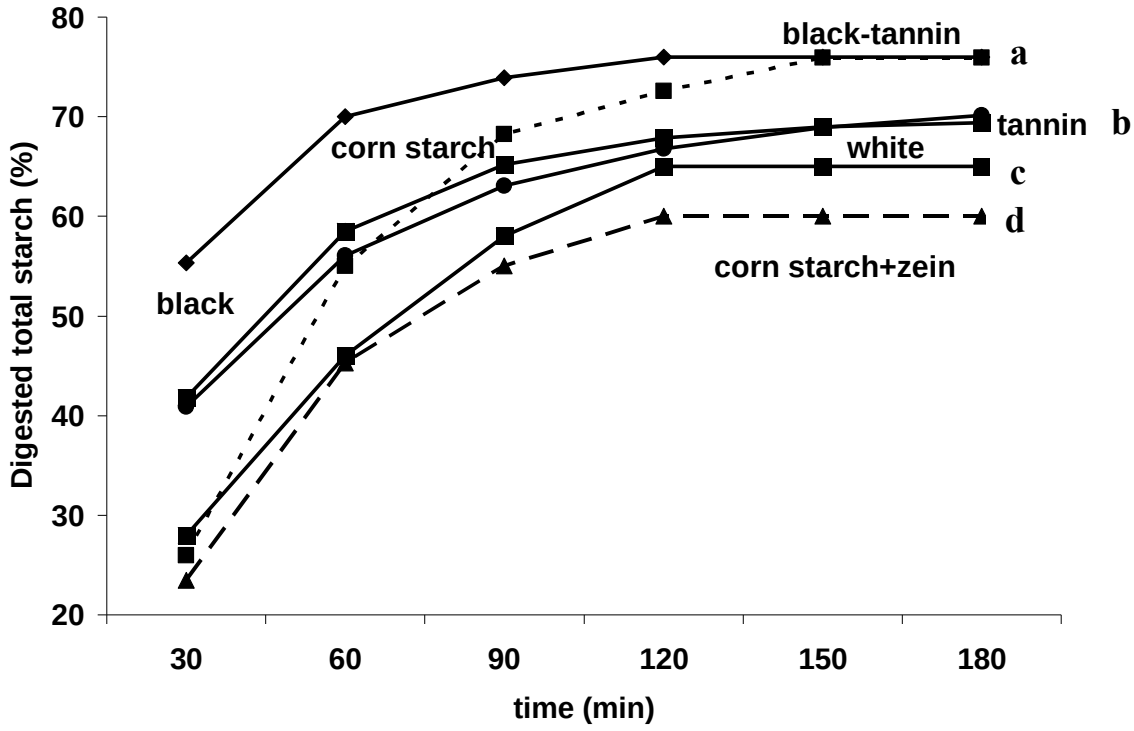

Fig. 42. Starch digestibility (%db) of double cooked corn starch porridges made with zein protein and 70% aqueous acetone sorghum bran extracts (corn starch: zein ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

Bran extracts rich in tannins and anthocyanins significantly ($p<0.05$) decreased starch digestibility of corn starch porridges (Fig. 43, Table B-V).

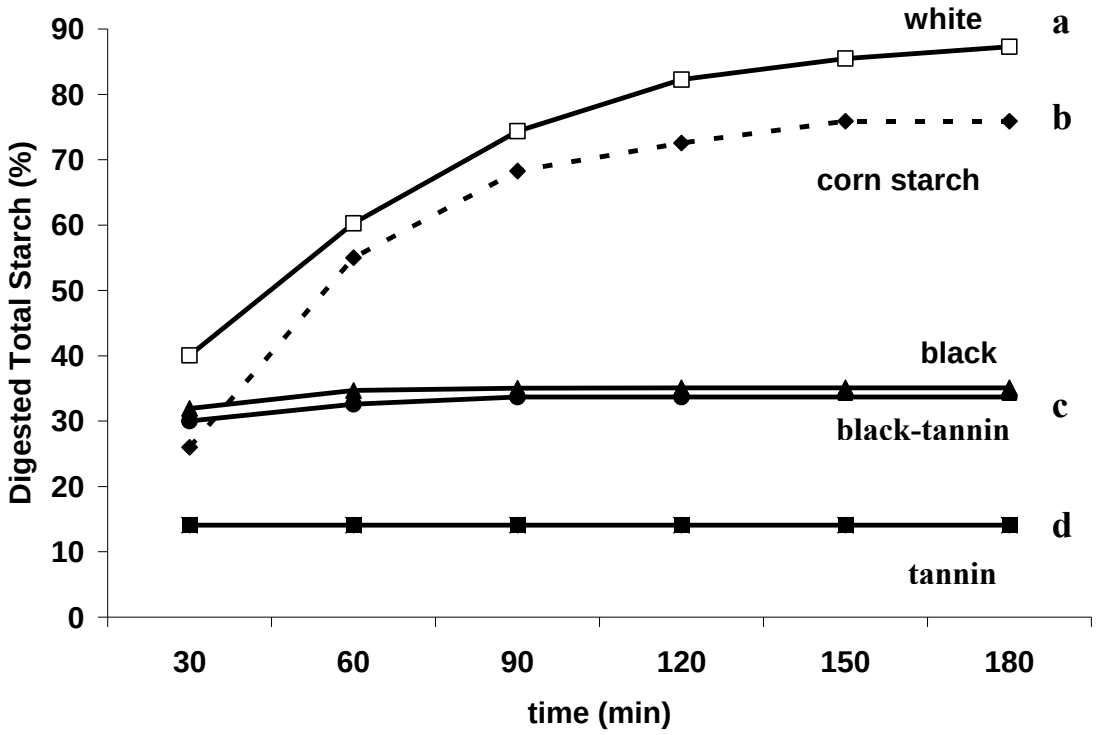

Fig. 43. Starch digestibility (%db) of 70% aqueous acetone sorghum bran extracts of double cooked corn starch porridges without zein. Values followed by the same letter are not significantly different ($p<0.05$).

Zein protein significantly ($p<0.05$) decreased EGIs of corn starch porridges. However, corn starch cooked with zein and white bran extract had significantly ($p<0.05$) lower EGIs than corn starch cooked with zein and specialty sorghum bran extracts.

When corn starch was cooked with only 70% aqueous acetone sorghum bran extracts, tannin, black with tannin and black sorghum bran extracts significantly ($p<0.05$) decreased EGIs of corn starch porridges (Fig. 44).

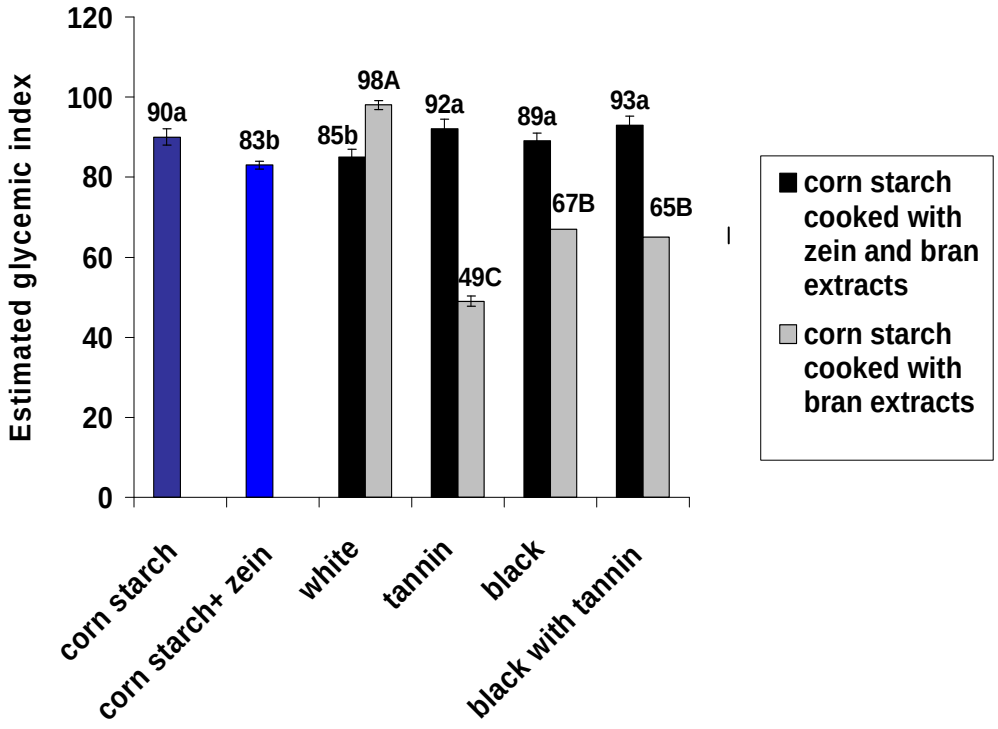

Fig. 44. EGIs of double cooked corn starch porridges made with zein protein and 70% aqueous acetone sorghum bran extracts (corn starch: zein ratio of 85:15) versus those made with only 70% aqueous acetone sorghum bran extracts. Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

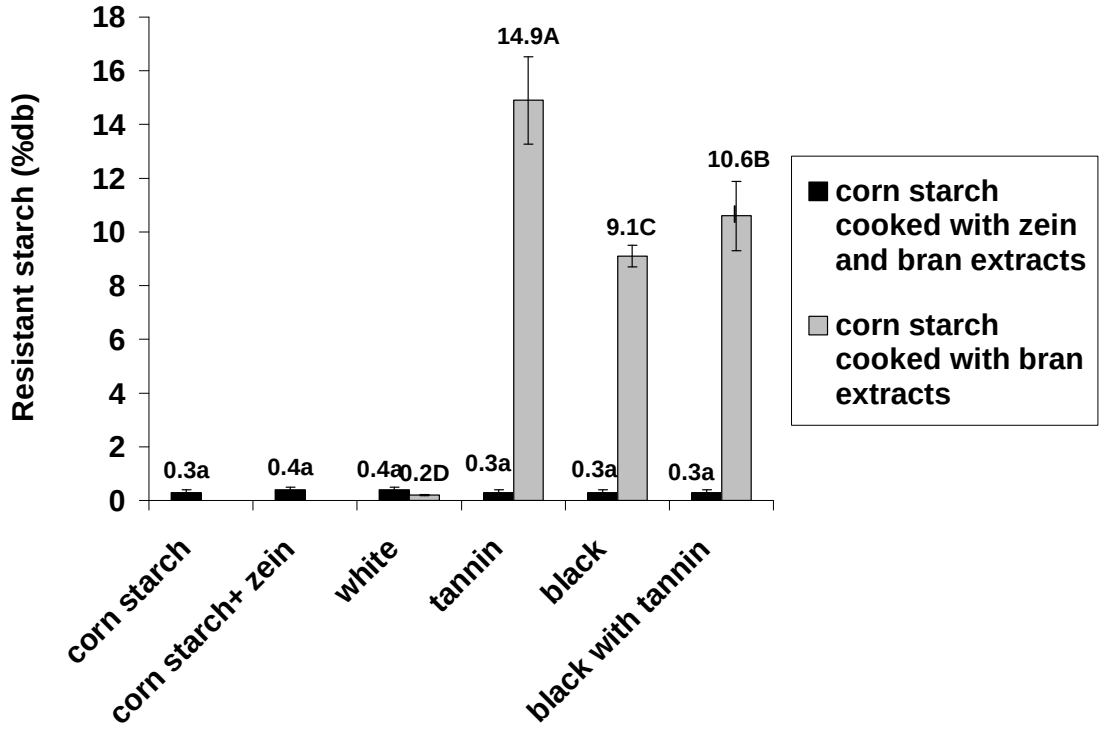

Fig. 45. RS contents of double cooked corn starch porridges made with zein protein and 70% aqueous acetone sorghum bran extracts (corn starch: zein ratio of 85:15) versus those made with only 70% aqueous acetone sorghum bran extracts. Values followed by the same uppercase letters are not significantly different ($p<0.05$), across all samples. Values followed by the same lowercase letters are not significantly different ($p<0.05$), across all samples.

Corn starch porridges cooked with black and black with tannin bran extracts had similar EGIs, while tannin bran extract porridges had the lowest EGIs (Fig. 44).

Specialty sorghum bran extracts significantly ($p<0.05$) increased RS contents of corn starch porridges (Fig. 45).

The Effects of Sorghum Bran Residue (remaining bran after 70% aqueous acetone extraction) on Double Cooked Starch Digestibility of Corn Starch Porridges

White sorghum bran residues significantly ($p<0.05$) increased starch digestibility, while tannin and black with tannin bran residue significantly ($p<0.05$) decreased starch digestibility of corn starch porridges. (Fig. 46).

Specialty sorghum bran residues seemed to inhibit starch digestibility by inhibiting digestive enzymes after 90 min of digestion (Fig. 46).

While black with tannin bran residue significantly ($p<0.05$) reduced EGIs of corn starch porridges, other bran residues did not significantly ($p<0.05$) affect the EGIs of corn starch porridges (Fig. 47). However, RS contents of corn starch porridges cooked with tannin bran residues had significantly ($p<0.05$) higher amounts than the other porridges (Fig. 48, Table B-VI).

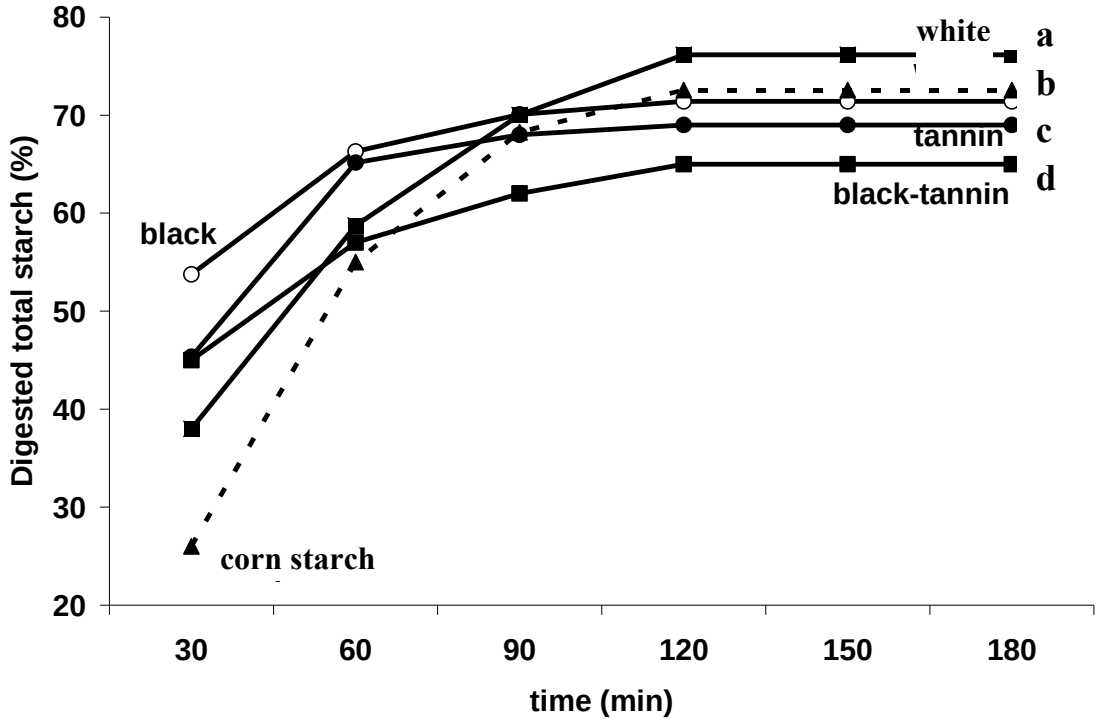

Fig. 46. Starch digestibility (%db) of double cooked corn starch porridges made with 70% aqueous acetone sorghum bran residue (corn starch: bran residue ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

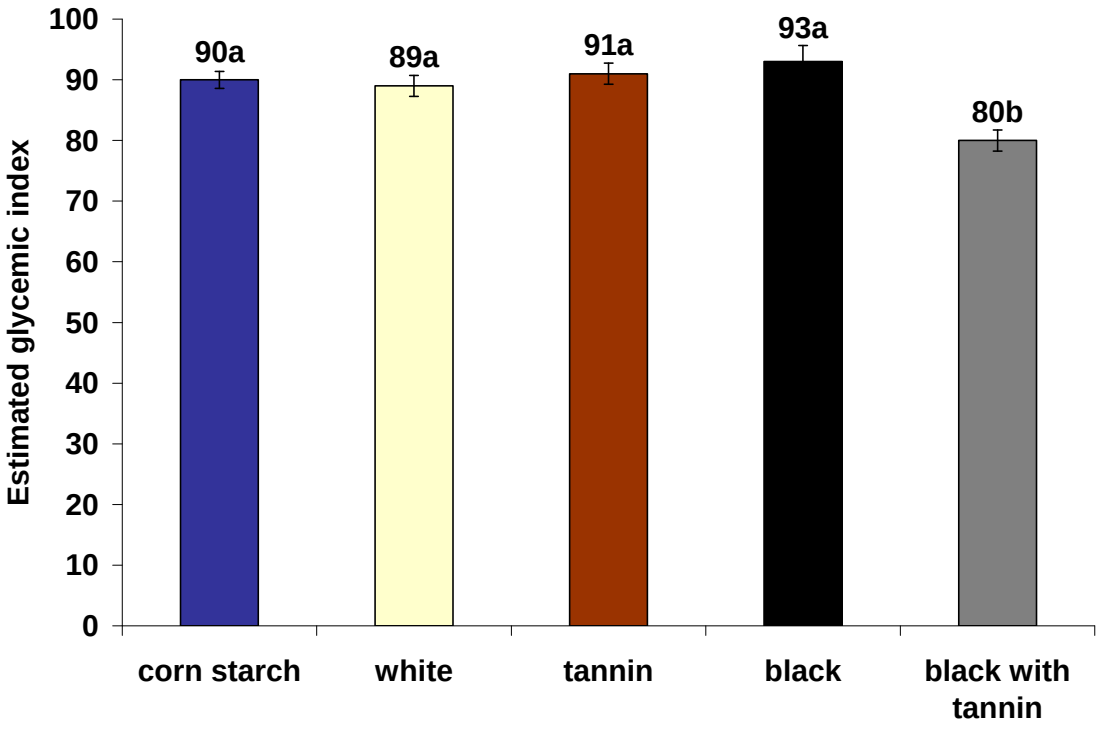

Fig. 47. EGIs of double cooked corn starch porridges made with 70% aqueous acetone sorghum bran residue (corn starch: bran residue ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

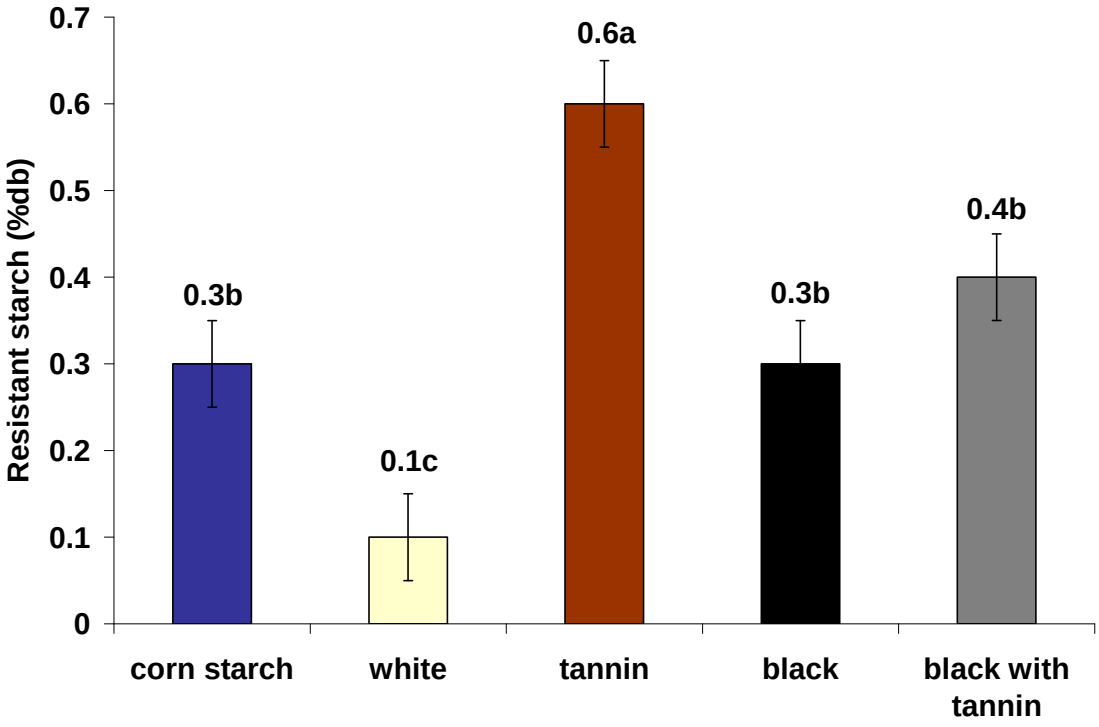

Fig. 48. RS (%db) of double cooked corn starch porridges made with 70% aqueous acetone sorghum bran residue (corn starch: bran residue ratio of 85:15). Values followed by the same letter are not significantly different ($p<0.05$).

The Effects of 70% Aqueous Acetone Sorghum Bran Extracts on Digestibility of Double Cooked High Amylose Corn Starch Porridges

High-maize 260 (high amylose corn) starch is resistant to stomach acids and digestive enzymes. Due to its high resistant starch content, hi-maize starch helps to manage blood glucose levels and balance energy between meals (National starch 2006). Sorghum bran extracts (except white bran) significantly ($p<0.05$) decreased overall starch digestibility, EGIs and increased RS contents of high amylose corn starch porridges. The most reduction in starch digestibility (Fig. 49, Table B-VII), EGIs (Fig. 50) and increase in RS (Fig. 51) of porridges were achieved with tannin bran extracts, followed by black with tannin and black bran extracts.

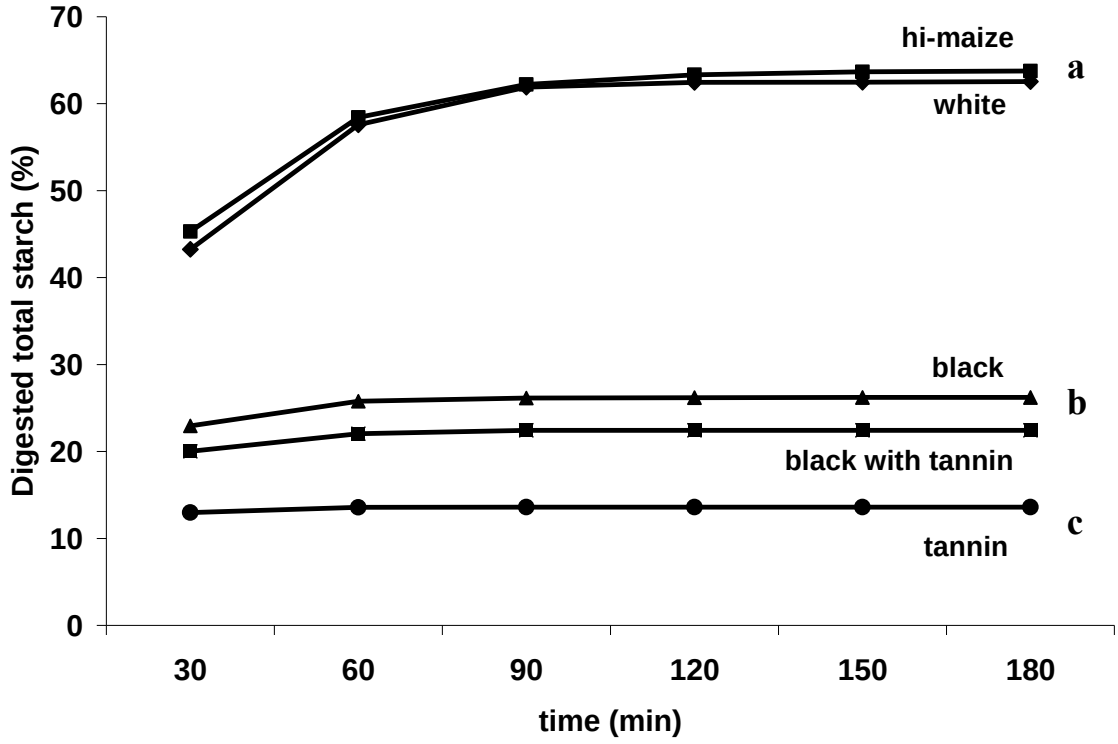

Fig. 49. Starch digestibility (%db) of double cooked high amylose corn starch porridges made with 70% aqueous acetone sorghum bran extracts. Values followed by the same letter are not significantly different ($p<0.05$).

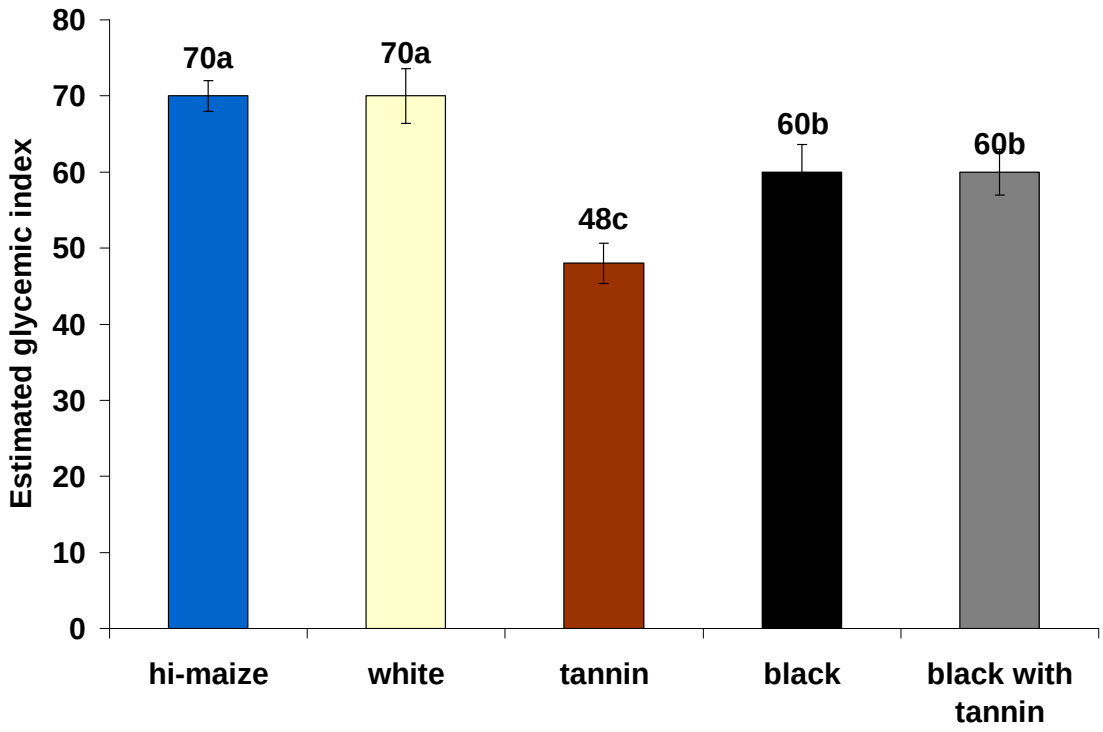

Fig. 50. EGIs of double cooked high amylose corn starch porridges made with 70% aqueous acetone sorghum bran extracts. Values followed by the same letter are not significantly different ($p<0.05$).

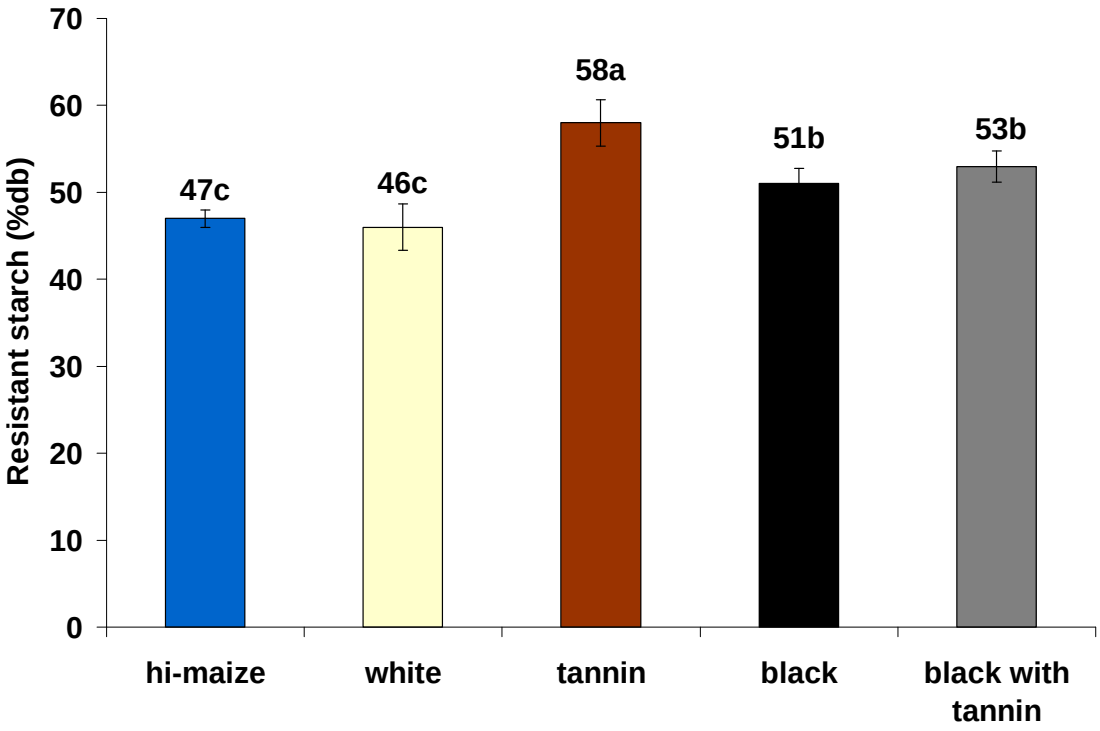

Fig. 51. RS (%db) of double cooked high amylose corn starch porridges made with 70% aqueous acetone sorghum bran extracts. Values followed by the same letter are not significantly different ($p<0.05$).

Discussion

Brans of the sorghum varieties contained approximately two fold more total phenols and condensed tannins than the whole sorghum grain, which shows that phenolic compounds in sorghum are highly concentrated in outer layers of the grain as reported (Awika and Rooney 2004, Beta et al 2000, Hahn et al 1984, Hahn and Rooney 1986).

Comparison of Starch Digestibility of Corn Starch Porridges Cooked with Sorghum Bran Extracts Obtained by Different Solvents

Since most of the phenolic compounds were concentrated in the bran of the sorghum varieties, extraction of phenols from bran was an effective way of concentrating these compounds.

Organic polar solvents were good choices to extract phenols from sorghum brans, while, distilled water was not effective. The highest solid contents of the bran extracts were obtained with 70% aqueous acetone for tannin and white brans and absolute methanol for black and black with tannin brans.

The coexistence of several biopolymers in the cell walls, their spatial organization, and the nature of interactions (cross-linking) among them might contribute to the mechanical strength, permeability, and solubility, and therefore to 70% aqueous acetone extraction yield. Sorghum bran extraction with 70% aqueous acetone removed most of the tannins.

The interaction of organic solvents with phenols occurs via van der Waals forces and hydrogen bonding (Dicko et al 2006). After removal of the solvent, the aqueous medium contained the phenols. More solids in the extracts were obtained from the brans of sorghum varieties with high total phenols and tannin contents.

Sorghum brans had between 25.5-52.7% of total starch. In general sorghum brans have approximately 30-40% fiber, 10% protein, 10% crude fat and 3% ash (Gordon 2001). During 70% aqueous acetone extraction, fiber and starch were intact. However, during extraction, some fats might have migrated into sorghum bran extracts. Therefore, sorghum bran extracts had phenols and some lipids.

The Effects of Double Cooking-cooling Cycles on Digestibility of Corn Starch Porridges

Corn starch gelatinizes between 62-72 ^{o}C with excessive water which ensures full starch gelatinization. Gelatinization is restricted if water is limiting (Tester et al 2004). Gelatinized starch becomes more susceptible to enzymes. In limited water systems, starch granules were slightly swollen and internal structure is partly intact (data not shown). Sievert and Pomeranz (1990) reported that starch: water ratio of 1: 3.5 (w/w) increased formation of RS. Our findings also showed that with two cooking-cooling cycles, starch: water ratio of 1: 3.5 (w/w) at first cooking was associated with a decrease in starch digestibility, and an increase in RS in porridges.

Upon cooling, amylose retrogradation occurs. However, when corn starch was cooked with reduced water (3 g starch in 12 ml water) most starch granules were gelatinized, with some intact granules. Addition of more distilled water (13 ml) at second cooking-cooling and with some bound water from first cooking-cooling, completed dispersion of gelatinized starch granules. We hypothesized that during the second cooking, retrograded amylose from the first cooking was melted and dispersed together with amylopectin in the porridges, and upon cooling more retrogradation took place, which increased the degree of crystallinity and RS formation in corn starch porridges. Double cooking increased RS and decreased starch digestibility and EGIs in the porridges.

The Effects of 70% Aqueous Acetone Sorghum Bran Extracts on Starch Digestibility of Double Cooked Whole Sorghum Grain Porridges

In sorghum endosperm, like other cereals, the starch granules and protein bodies are embedded in a continuous protein matrix in the peripheral and corneous areas. Some researchers (Hamaker and Bugusu 2003, Zhang and Hamaker 1998, Duodu et al 2003) observed that sorghum kafirins formed resilient web or sheet-like structures due to formation of disulfide cross links within and possibly between, protein bodies during cooking. This interferes with accessibility of gelatinized starch by digestive enzymes. This study showed that whole sorghum porridges had significantly ($p<0.05$) lower starch

digestibility than whole corn porridges. Whole tannin, black, and black with tannin sorghum porridges had significantly lower starch digestibility than whole white sorghum porridges.

Porridges were prepared under high moisture (85-88%), temperatures (≤ 95 ^{o}C), and high shear. Mixing, high moisture and high shear enhance molecular interactions, particularly those of condensed tannins with protein and other phenolic compounds (Ngwenya 2007). Thus protein denatured by cooking had open loose structures which promoted phenols-protein interactions. The hydrophobic interactions likely predominated at elevated temperatures during porridge cooking. When the porridge cooled, hydrogen bonds were re-formed, and reinforced the hydrophobic interactions as mentioned by Verge et al (2002).

After cooking, pepsin and alpha-amylase digestion were applied to sub-sampled porridges during starch digestion assays. Pepsin preferentially cleaves peptide bonds with aromatic amino acids (Schnaith 1989), thus effectively disrupting hydrophobic regions of the protein, phenolic compounds, specifically tannins may be released into solution during enzyme digestion. This could inhibit alpha amylase activity, resulting in decreased starch digestibility, EGI, and increased RS contents of the porridges.

During pepsin digestion, de-polymerization of tannins may occur due to low pH pepsin buffer (KCl-HCl, pH 1.5). The free depolymerized polymers of tannins inhibited alpha amylase and interacted with the other phenolics such as anthocyanins. Then when alpha amylase was added to the porridges, higher pH of the amylase buffer (Tris maleate buffer, pH 6.9) caused polymerization (Porter 1992) of the phenols and tannins. At the same time, alpha-amylase hydrolyzed gelatinized starch, and may have released some phenols entrapped in the food matrix. Consequently, phenolic compounds mostly inhibited alpha-amylase, and lowered starch digestibility, EGI and increased RS contents of the porridges.

Black with tannin sorghum porridges had lower starch digestibility than tannin and black sorghum porridges. Since, in aqueous environments and in the presence of amylase buffer with high pH, polymerization occurs between tannin molecules or with

other phenolics such as anthocyanins (Remy et al 2000, Wieser 2007), the combined effects of tannins and anthocyanins on enzyme inhibition likely lowered starch digestibility more.

To fortify our findings that phenols decreased starch digestibility in whole sorghum porridges, we cooked whole sorghum grains with their corresponding bran extracts. Specialty sorghum bran extracts significantly ($p<0.05$) decreased starch digestibility of whole sorghum porridges even more ($p<0.05$). One hypothesis could be that the excessive amounts of free phenols would increase enzyme inhibition, interactions/entangling with soluble gelatinized starch and denatured protein. This would result in decreased starch digestibility, EGI, and increased RS contents of whole sorghum porridges cooked with specialty sorghum bran extracts. As mentioned before, at high pH, during alpha-amylase digestion, polymerization of phenolic compounds could occur.

To understand if phenols were inhibiting the enzymes, we applied increasing concentrations of alpha amylase during starch digestion to corn starch porridges cooked with 70% aqueous acetone sorghum bran extracts. Increased enzyme concentration caused higher starch digestibility of the porridges at 30 min of digestion (RDS), however, after that it remained constant over the digestion period (data not shown). This clearly showed that phenols in sorghum varieties inhibit enzyme activity.

The higher amounts of phenolics in sorghum varieties resulted in higher amounts of RS content.

The Effects of Zein Protein on Starch Digestibility of Double Cooked Corn Starch Porridges Made with 70% Aqueous Acetone Sorghum Bran Extracts

Zein in corn is similar to kafirins in sorghum, because they both are high in proline residues (Kitts and Weiler 2003). Thus zein was used to investigate the effect of sorghum phenols in the presence of prolamin proteins.

Re-association of gelatinized starch occurs almost immediately after gelatinization. Stable hydrogen bonding between linear segments of amylose occur post gelatinization in most food processes (Wu et al 1992), and polymers of gelatinized starch

immediately associate with other components in the food system. This often decreases solubilization and increases enzyme resistance of starch. In the presence of zein, corn starch interacted with zein upon gelatinization, resulting in significantly ($p<0.05$) lower starch digestibility and EGI of the porridges without changing RS content of the corn starch porridges.

Phenols have strong affinity for proteins high in proline content like the prolamins (Scalbert et al 2000, Emmambux and Taylor 2003).

When specialty sorghum bran extracts were used to cook corn starch and zein. Phenols preferentially interacted with zein proteins which led to increased starch digestibility. High temperature (up to 95 ^{o}C) and shear, generated during porridge cooking, denatured protein, likely reduced protein-protein and starch-protein associations, thus facilitating zein interactions with phenolic polymers, which increased accessibility of gelatinized starch by enzymes. In the absence of phenolic compounds (white sorghum bran extract), zein encapsulated gelatinized starch, making it less susceptible to enzymes. This resulted in lower starch digestibility and EGI than corn starch cooked with only white bran extracts.

The combination of starch-phenols-zein in the system overcame the effect of phenols on starch digestibility, because zein complexed with the phenols.

The Effects of Sorghum Bran Residue (remaining bran after 70% aqueous acetone extraction) on Starch Digestibility of Corn Starch Porridges

Specifically, corn starch porridges made with black sorghum bran residues had significantly ($p<0.05$) higher RDS than the porridges made with other sorghum bran residues. This was caused by the larger particle size and different physical structure of black sorghum brans, which were visually supported by bright field microscopy. Soluble fiber has ability to decrease enzyme diffusion by increasing viscosity of the surroundings. Since sorghum bran is mainly insoluble fiber (Gordon 2001), the larger particles of black brans interfered with re-association of starch chains, causing weaker gels. This increased diffusion of the enzymes into porridges, producing higher starch digestibility.

While black with tannin bran residue significantly ($p<0.05$) reduced EGIs of corn starch porridges, other bran residues did not significantly ($p<0.05$) affect EGIs of corn starch porridges. Combined effect of tannins and anthocyanins caused lower starch digestibility and EGI of the corn starch porridges.

Enzyme inhibition was observed after 90 min of digestion. This was due to enzyme inhibition by phenolic compounds (with 70% aqueous acetone extraction) in the bran residues. However, RS contents of corn starch porridges were not significantly ($p<0.05$) affected by sorghum bran residues.

The Effects of 70% Aqueous Acetone Sorghum Bran Extracts on Digestibility of Double Cooked High Amylose Corn Starch Porridges

Starch digestibility and EGIs significantly ($p<0.05$) decreased; RS contents significantly ($p<0.05$) increased in porridges cooked with 70% aqueous acetone sorghum bran extracts, except white bran extract.

High amylose corn starch (resistant starch) has been shown to help manage blood glucose levels and balance energy between meals. Typically, with heat and excess water, the semi-crystalline granules of normal starch will gelatinize, hydrate, and swell, producing a viscous paste. Gelatinization of hi-amylose starch occurs only under pressure cooking (>100 ^{o}C). The RVA used in this study had a maximum temperature of 95-100 ^{o}C. So high amylose corn starch did not gelatinize and viscosity did not develop.

In vivo studies showed that the replacement of 20% of the flour in bread with high amylose corn starch reduced the post-prandial plasma glucose level by 45% (National Starch 2006). Replacing high amylose corn starch with flour and using phenolic rich sorghum bran extracts as liquid medium in those products would increase health benefits of highly resistant high amylose corn starch to individuals with obesity and diabetes.

Overall, the phenols of specialty sorghum varieties used in this study significantly affect the rate and extent of starch digestibility of porridges. Significantly ($p<0.05$) lower EGI and higher RS values were observed in porridges made with bran extracts containing high levels of phenols compounds. These results demonstrate that the

sorghum phenols, present mainly in the pericarp, have potential applications in food and pharmaceutical industries to decrease health problems related to type 2 diabetes and to reduce weight because of its reduced hydrolysis.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Phenolic compounds in the pericarp and testa of specialty sorghum varieties provided a unique opportunity to produce low EGI and high RS-containing palatable porridges with natural, attractive dark purple and red colors, with high levels of dietary fiber, and antioxidants. Whole sorghum grains had 3-22.5 mg/g gallic acid total phenols and 0-34.4 mg catechin eq./g tannin contents. Their brans had 4.3-40.3 mg/g gallic acid total phenols and 0-75 mg catechin eq./g tannin contents.

Seventy percent (70%) aqueous specialty sorghum bran extracts significantly ($p<0.05$) decreased starch digestibility of corn starch, enzyme resistant high amylose corn starch and whole sorghum porridges. The extracts significantly ($p<0.05$) decreased EGI values by 33%, 21%, 20% and increased RS contents by 1100%, 300%, and 15% of corn starch, whole sorghum, and high amylose corn starch porridges, respectively (Table III).

Tannin sorghum varieties (hi-tannin and black with tannin) were most effective for lowering starch digestibility, and EGI and increasing RS contents of the porridges, when used as whole grains, brans, and bran extracts.

Combined effects of tannins and anthocyanins in black with tannin sorghum variety significantly ($p<0.05$) decreased starch digestibility, and EGI, and increased RS contents of whole sorghum grain and bran added to endosperm porridges even more than other specialty sorghum varieties. However, black bran structure and its larger particle size distribution prevented anthocyanins from reducing EGI of porridges when they were added to hard and soft endosperm flours in the form of brans.

All whole sorghum porridges had significantly ($p<0.05$) lower starch digestibility and EGI values than whole white corn porridges.

Table III

EGI and RS Values of Different Foods Compared to Porridges Used in This Study*

Porridges Used and Cooked Foods	EGI (average)	RS (average)
High amylose Corn Starch Porridges cooked with water	**70**	**47.0**
High-maize (high amylose corn) starch corn starch Porridges cooked with specialty sorghum bran extracts	**56**	**54.0**
Double cooked corn starch porridges (water)	**90**	**1.0**
Corn starch cooked with specialty sorghum bran extracts	**60**	**12.0**
Whole grain corn porridges cooked with water	**100**	**0.1**
Whole specialty sorghum porridges cooked with water	**87**	**1.2**
Whole specialty sorghum porridges cooked with specialty sorghum bran extracts	**69**	**4.8**
hard endosperm cooked with water	**99**	**0.2**
hard endosperm cooked with specialty sorghum bran (water)	**99**	**0.4**
soft endosperm cooked with water	**103**	**0.2**
soft endosperm cooked with specialty sorghum brans (water)	**100**	**0.6**
Lentils*	42	6.8
Chickpeas*	47	4.4
Beans*	60	5.5
Spagetti*	78	2.9
Boiled potatoes*	99	1.0

* National Starch (2006).

When corn starch was cooked with zein protein in specialty sorghum bran extracts, zein complexed with the phenolic compounds, consequently, gelatinized starch was more available for the enzymes. This increased starch digestibility and EGI and decreased RS contents of the corn starch porridges. When we took each structural component (starch-protein-phenolics) apart in whole sorghum grain and cook together,

the effect of phenolic compounds was different on the starch digestibility compared to when they were cooked as whole grains. The other components of the grain such as, germ, peripheral endosperm and aleurone layer might affect starch digestibility of the whole sorghum porridges.

Consumption of resistant starch is approximately 3-6 grams/day in the typical American diet (National Starch 2006). Natural high amylose resistant starch is becoming a popular food ingredient for flour substitution to increase insoluble fiber content of foods. The replacement of 20% of the flour in bread with high amylose corn starch (Hi-maize 260) reduced the post-prandial plasma glucose level by 45% (National Starch 2006). In our study, the amounts of RS in the porridges were strongly related to the amounts of phenols in sorghum varieties and their bran extracts.

Different plant extracts have been used to reduce oxidative rancidity and improve shelf life of some meat and oil products (Koleva et al 2001). In the past, tannin brans were added to breads, tortillas, cookies, extrudates, and meat products for the same purpose as partial or complete substitutes for other cereals.

However, this research distinguished specialty sorghum brans from the other sorghum varieties and white corn that their extracts have potentials to be used in starchy/sugary foods to reduce EGI and to increase RS contents to provide healthy benefits to individuals with obesity, diabetes, and healthy individuals as well.

Suggested future works should include:

- *In vitro* methods to measure GI may prove helpful for the initial screening of specialty sorghum varieties or for the industrial development of foods; however, in accordance with the definition, the GI must be confirmed *in vivo* by clinical trials.
- Screening other specialty sorghum varieties with phenolic compounds in a diversity of foods used as whole grains, brans and/or their bran extracts would help to better understand the effect of sorghum phenols on starch digestibility.
- Removing fats from sorghum bran by hexane will eliminate the effect of lipids on starch digestibility.
- Aqueous bran extracts could be further concentrated by freeze-drying and obtained as fine powder. This would permit use of the same amounts of the extracts from each type of bran in foods. However, incorporating into liquid systems can be challenging.
- Developing an efficient milling procedure for sorghum brans to eliminate the structural differences to understand bran effects on starch digestibility of porridges.

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APPENDIX A

THE EFFECTS OF SPECIALTY SORGHUM BRAN ON STARCH DIGESTIBILITY OF SOFT AND HARD SORGHUM ENDOSPERM FLOUR PORRIDGES

TABLE A-I

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Pure Ground Endosperm Porridges*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf***	**k***	**RDS***	**SDS***
Hard endosperm	**65.1a**	**76.2a**	**77.2a**	**78a**	**78a**	**78a**	**78a**	**0.06a**	**65.1a**	**78a**
Soft endosperm	**62.4a**	**79.4a**	**84.7b**	**86.4b**	**87.02b**	**87.2b**	**87.2b**	**0.05a**	**62.4a**	**87.2b**

***Values within each column with different letters are significantly different at P<0.05.**

***Cinf: the equilibrium percentage of starch hydrolyzed after 180 min, k: the kinetic constant, RDS: rapidly digested starch, SDS: slowly digested starch.**

TABLE A-II

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Pure Ground White Endosperm (Hard Endosperm) Cooked with Sorghum Brans*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Hard endosperm	**65.1a**	**76.2a**	**77.2c**	**78c**	**78c**	**78c**	**78c**	**0.06a**	**65.1a**	**78c**
Hard endosperm +tannin bran	**64.9a**	**71.9b**	**72.8d**	**72.9d**	**72.9d**	**72.9d**	**72.9d**	**0.08a**	**64.9a**	**72.9d**
Hard endosperm+ white bran	**72.4b**	**78a**	**81b**	**82b**	**82b**	**82b**	**82b**	**0.30b**	**72.4b**	**82b**
Hard endosperm+ black bran	**73.2b**	**94.3c**	**99a**	**100a**	**100a**	**100a**	**100a**	**0.04c**	**73.2b**	**100a**
Hard endosperm+ black with tannin bran	**54.9c**	**67.4d**	**70.4e**	**71.2d**	**71.2d**	**71.2d**	**71.2d**	**0.03c**	**54.9c**	**71.2d**
LSD	**0.9**	**1.8**	**1.0**	**1.8**	**1.8**	**1.8**	**1.8**	**0.04**	**0.9**	**1.8**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE A-III

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Each Type of Pure Ground Sumac Endosperm (Soft Endosperm) Cooked with Sorghum Brans*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Soft endosperm	**62.4c**	**79.4c**	**84.7c**	**86.4c**	**86.4c**	**86.4c**	**86.4c**	**0.05a**	**62.4c**	**86.4c**
Soft endosperm+ tannin bran	**68.7a**	**74.3d**	**74.8d**	**74.9d**	**74.9d**	**74.9d**	**74.9d**	**0.08a**	**68.7a**	**74.9d**
Soft endosperm + white bran	**76.7d**	**85b**	**88b**	**90b**	**90b**	**90b**	**90b**	**0.33a**	**76.7d**	**90b**
Soft endosperm + black bran	**65.7b**	**88.2a**	**96a**	**98.6a**	**98.6a**	**98.6a**	**98.6a**	**0.04a**	**65.7b**	**98.6a**
Soft endosperm + black with tannin	**45e**	**61.4e**	**67.8e**	**70.5e**	**70.5e**	**70.5e**	**70.5e**	**0.05a**	**45e**	**70.5e**
LSD	**1.3**	**1.1**	**0.9**	**1.6**	**1.6**	**1.6**	**1.6**	**0.03**	**1.3**	**1.6**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE A-IV

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Each Type of Porridges*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Hard endosperm + black with tannin bran	**54.9c**	**67.4c**	**70.4c**	**71.2c**	**71.2c**	**71.2c**	**71.2c**	**0.03b**	**54.9c**	**71.2c**
Soft endosperm +black with tannin bran	**45.0d**	**61.4d**	**67.8d**	**70.5c**	**70.5c**	**70.5c**	**70.5c**	**0.05a**	**45.0d**	**70.5c**
Hard endosperm	**65.1b**	**76.2b**	**77.2b**	**78.0b**	**78.0b**	**78.0b**	**78.0b**	**0.06a**	**65.1b**	**78.0b**
Soft endosperm	**62.4a**	**79.4a**	**84.7a**	**86.4a**	**86.4a**	**86.4a**	**86.4a**	**0.05a**	**62.4a**	**86.4a**
LSD	**0.9**	**1.0**	**0.6**	**1.5**	**1.5**	**1.5**	**1.5**	**0.01**	**0.9**	**1.5**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE A-V

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Each Type of Porridges*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Hard endosperm + tannin bran	**64.9b**	**71.9d**	**72.8d**	**72.9d**	**72.9d**	**72.9d**	**72.9d**	**0.08a**	**64.9b**	**72.9d**
Soft endosperm +tannin bran	**68.7a**	**74.3c**	**74.8c**	**74.9c**	**74.9c**	**74.9c**	**74.9c**	**0.08a**	**68.7a**	**74.9c**
Hard endosperm	**65.1b**	**76.2b**	**77.2b**	**78.0b**	**78.0b**	**78.0b**	**78.0b**	**0.06a**	**65.1b**	**78.0b**
Soft endosperm	**62.4c**	**79.4a**	**84.7a**	**86.4a**	**86.4a**	**86.4a**	**86.4a**	**0.05a**	**62.4c**	**86.4a**
LSD	**1.2**	**0.9**	**0.8**	**0.9**	**0.9**	**0.9**	**0.9**	**0.0**	**1.2**	**0.9**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE A-VI

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Each Type of Porridges*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Hard endosperm + black bran	**73.2a**	**94.3a**	**99a**	**100a**	**100a**	**100a**	**100a**	**0.04a**	**73.2a**	**100a**
Soft endosperm + black bran	**65.7b**	**88.2b**	**96b**	**98.6a**	**98.6a**	**98.6a**	**98.6a**	**0.04a**	**65.7b**	**98.6a**
Hard endosperm	**65.1b**	**76.2d**	**77.2d**	**78.0c**	**78.0c**	**78.0c**	**78.0c**	**0.06a**	**65.1b**	**78.0c**
Soft endosperm	**62.4c**	**79.4c**	**84.7c**	**86.4d**	**86.4d**	**86.4d**	**86.4d**	**0.05a**	**62.4c**	**86.4d**
LSD	**0.7**	**1.0**	**0.7**	**1.5**	**1.5**	**1.5**	**1.5**	**0.02**	**0.7**	**1.5**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE A-VII

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Each Type of Porridges*

			time (min)							
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Hard endosperm + white bran	**72.4b**	**78c**	**81c**	**82c**	**82c**	**82c**	**82c**	**0.30b**	**72.4b**	**82c**
Soft endosperm + white bran	**76.7a**	**85a**	**88a**	**90a**	**90a**	**90a**	**90a**	**0.33b**	**76.7a**	**90a**
Hard endosperm	**65.1c**	**76.2c**	**77.2d**	**78d**	**78d**	**78d**	**78d**	**0.06a**	**65.1c**	**78d**
Soft endosperm	**62.4d**	**79.4b**	**84.7b**	**86.4b**	**86.4b**	**86.4b**	**86.4b**	**0.05a**	**62.4d**	**86.4b**
LSD	**2.1**	**1.9**	**1.2**	**1.1**	**1.1**	**1.1**	**1.1**	**0.03**	**2.1**	**1.1**

***Values within each column with different letters are significantly different at P<0.05.**

APPENDIX B

EFFECTS OF SORGHUM *Sorghum bicolor* (L.) Moench PHENOLICS ON RESISTANT STARCH (RS) and STARCH DIGESTIBILITY OF PORRIDGES

TABLE B-I

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Double Cooked Corn Starch Porridges*

	Time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Single cooked corn starch	**35.9a**	**58.4a**	**72.6a**	**81.5a**	**81.5a**	**81.5a**	**81.5a**	**0.02a**	**35.9a**	**81.5a**
Double cooked corn starch (25 ml water at 1. cooking)	**30b**	**55.4b**	**69b**	**78b**	**78b**	**78b**	**78b**	**0.02a**	**30b**	**78b**
Double cooked corn starch (12 ml water at 1. cooking)	**26c**	**55b**	**68.3b**	**72.6c**	**72.6c**	**72.6c**	**72.6c**	**0.02a**	**26c**	**72.6c**
LSD	**2.0**	**1.3**	**1.8**	**1.9**	**1.9**	**1.9**	**1.9**	**0.0**	**2.0**	**1.9**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE B-II

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for double cooked ground whole sorghum porridges with distilled water*

	Time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Whole corn	**61.6a**	**75.0a**	**78a**	**78.7a**	**78.7a**	**78.7a**	**78.7a**	**0.05c**	**61.6a**	**78.7a**
Whole white	**58.2b**	**66.0b**	**67.8b**	**68.3b**	**68.3b**	**68.3b**	**68.3b**	**0.07c**	**58.2b**	**68.3b**
Whole high tannin	**58.1b**	**62.7cd**	**63.2c**	**63.2c**	**63.2c**	**63.2c**	**63.2c**	**0.1a**	**58.1b**	**63.2c**
Whole black	**59.6c**	**63.7c**	**64c**	**64c**	**64c**	**64c**	**64c**	**0.1b**	**59.6c**	**64c**
Whole black with tannin	**51.3d**	**60.9d**	**62.8c**	**63.3c**	**63.3c**	**63.3c**	**63.3c**	**0.06c**	**51.3d**	**63.3c**
LSD	**1.4**	**2.5**	**2.4**	**2.0**	**2.0**	**2.0**	**2.0**	**0.02**	**1.4**	**2.0**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE B-III

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for double cooked ground whole sorghum porridges with 70% aqueous Acetone Sorghum Bran Extracts*

	Time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Whole tannin + tannin bran extract	**20.8c**	**28.9d**	**32.3d**	**33.8d**	**33.8d**	**33.8d**	**33.8d**	**0.02b**	**20.8c**	**33.8d**
Whole black + black bran extract	**27.3c**	**42.1c**	**49.7c**	**51.8c**	**51.8c**	**51.8c**	**51.8c**	**0.08a**	**27.3c**	**51.8c**
Whole corn+ corn bran extract	**62.0a**	**77.0a**	**80a**	**80.0a**	**80.0a**	**80.0a**	**80.0a**	**0.05a**	**62.0a**	**80.0a**
Whole black with tannin + black with tannin bran extract	**27.0c**	**40.0c**	**48.0c**	**48.0c**	**48.0c**	**48.0c**	**48.0c**	**0.03a**	**27.0c**	**48.0c**
Whole white + white bran extract	**57.0a**	**65.5b**	**66.0b**	**66.5b**	**66.5b**	**66.5b**	**66.5b**	**0.07a**	**57.0a**	**66.5b**
LSD	**8.0**	**8.0**	**8.0**	**7.7**	**7.7**	**7.7**	**7.7**	**0.03**	**8.0**	**7.7**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE B-IV

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Double Cooked Corn Starch Porridges Made with Zein Protein and 70% queous Acetone Sorghum Bran Extracts*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Double cooked corn starch	**26.0c**	**55c**	**68.3b**	**72.6a**	**72.6a**	**72.6a**	**72.6a**	**0.02c**	**26.0c**	**72.6a**
Corn starch +zein	**23.5d**	**45.3d**	**55f**	**60.0d**	**60.0d**	**60.0d**	**60.0d**	**0.10a**	**23.5d**	**60.0d**
Corn starch + zein + white bran extract	**28.2c**	**46d**	**58e**	**65.0c**	**65.0c**	**65.0c**	**65.0c**	**0.10a**	**28.2c**	**65.0c**
Corn starch + zein + tannin bran extract	**40.9b**	**56.1c**	**63.1d**	**66.8bc**	**66.8bc**	**66.8bc**	**66.8bc**	**0.03bc**	**40.9b**	**66.8bc**
Corn starch + zein + black bran extract	**41.8b**	**58.5b**	**65.2c**	**67.9b**	**67.9b**	**67.9b**	**67.9b**	**0.03bc**	**41.8b**	**67.9b**
Corn starch +zein + black with tannin extract	**55.3a**	**70a**	**73.9a**	**74.1a**	**74.1a**	**74.1a**	**74.1a**	**0.04b**	**55.3a**	**74.1a**
LSD	**2.5**	**1.9**	**1.9**	**2.1**	**2.1**	**2.1**	**2.1**	**0.01**	**2.5**	**2.1**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE B-V

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Double

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Double cooked corn starch	**26c**	**55b**	**68.3b**	**72.6b**	**72.6b**	**72.6b**	**72.6b**	**0.02b**	**26.0c**	**72.6b**
Corn starch+ white bran extract	**40.1a**	**60.3a**	**74.4a**	**82.3a**	**82.3a**	**82.3a**	**82.3a**	**0.03b**	**40.1a**	**82.3a**
Corn starch+ black bran extract	**32b**	**34.7c**	**35.0c**	**35.1c**	**35.1c**	**35.1c**	**35.1c**	**0.8a**	**32b**	**35.1c**
Corn starch+ black with tannin bran extract	**30.0b**	**32.6c**	**33.7c**	**33.7c**	**33.7c**	**33.7c**	**33.7c**	**0.8a**	**30.0b**	**33.7c**
Corn starch+ tannin bran extract	**14.1d**	**14.1d**	**14.1d**	**14.1d**	**14.1d**	**14.1d**	**14.1d**	**0.8a**	**14.1d**	**14.1d**
LSD	**3.3**	**3.2**	**3.2**	**4.2**	**4.2**	**4.2**	**4.2**	**0.2**	**3.3**	**4.2**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE B-VI

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Double Cooked Corn Starch Porridges Made with 70%aqueous Acetone Sorghum Bran Residues*

	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
Corn starch+ tannin bran residue	**45.3b**	**65b**	**68.0ba**	**69c**	**69c**	**69c**	**69c**	**0.02a**	**45.3b**	**69c**
Corn starch+ black bran residue	**53.8a**	**66.3a**	**70.1a**	**71.4b**	**71.4b**	**71.4b**	**71.4b**	**0.03a**	**53.8a**	**71.4b**
Corn starch+ black with tannin bran residue	**45.3b**	**57.0d**	**62.0ca**	**65d**	**65d**	**65d**	**65d**	**0.05a**	**45.3b**	**65d**
Corn starch+ white bran residue	**38c**	**58.7c**	**70.0a**	**76.2a**	**76.2a**	**76.2a**	**76.2a**	**0.02a**	**38c**	**76.2a**
Double cooked corn starch	**26d**	**55.0e**	**68.2b**	**72.6b**	**72.6b**	**72.6b**	**72.6b**	**0.02a**	**26d**	**72.6b**
LSD	**1.4**	**1.2**	**1.5**	**1.8**	**1.8**	**1.8**	**1.8**	**0.02**	**1.4**	**1.8**

***Values within each column with different letters are significantly different at P<0.05.**

TABLE B-VII

Percentage (db) of Starch Hydrolyzed at Different Times (min), and Calculated C∞ and k Constants for Double Cooked High Amylose Corn Starch Porridges made with 70% aqueous Acetone Sorghum Bran Extracts*

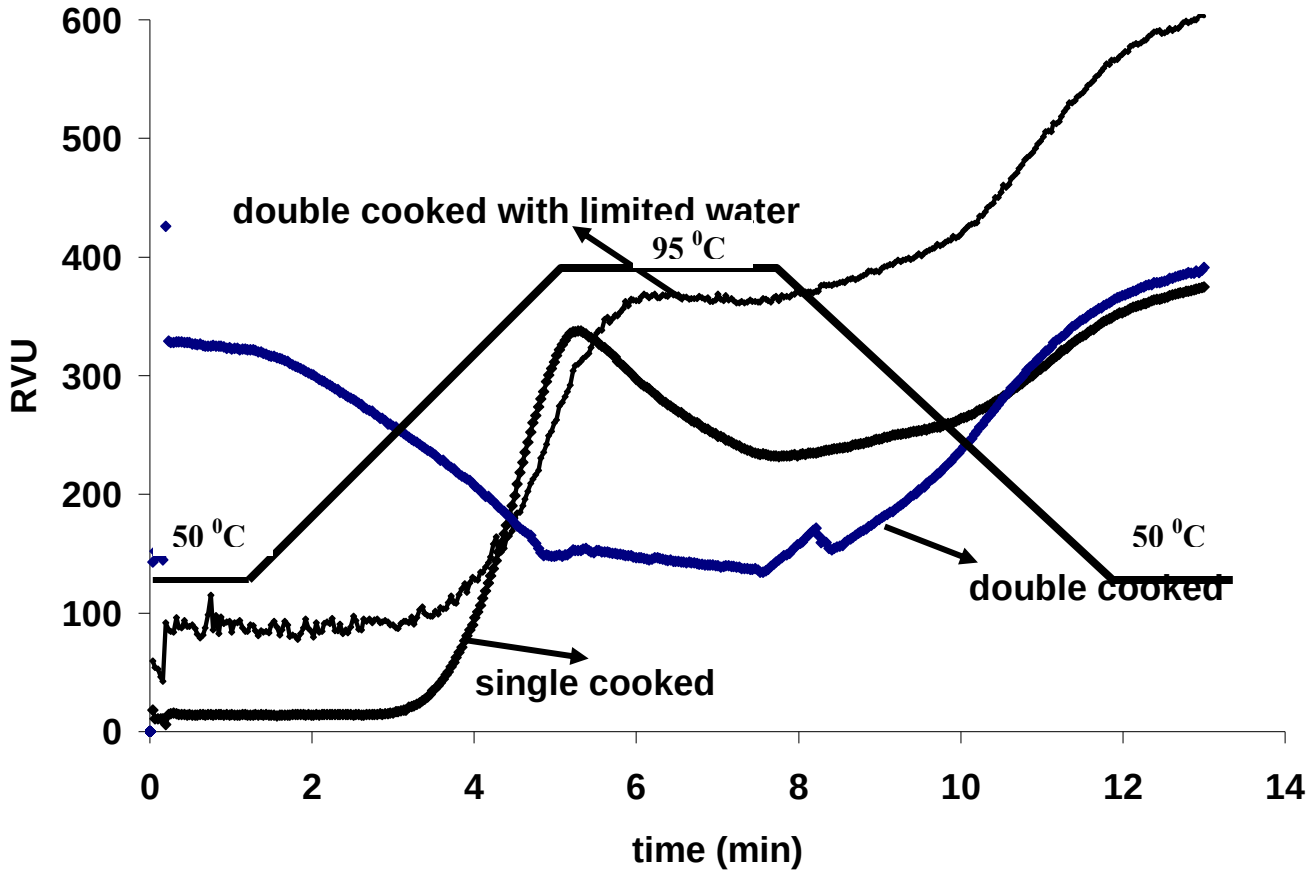
	time (min)									
Porridges	**30**	**60**	**90**	**120**	**150**	**180**	**Cinf**	**k**	**RDS**	**SDS**
High amylose corn starch	**44a**	**59a**	**60a**	**60a**	**60a**	**60a**	**60a**	**0.04a**	**44a**	**60a**
High amylose corn starch + white bran extract	**42a**	**59a**	**60a**	**60a**	**60a**	**60a**	**40a**	**0.08a**	**42a**	**60a**
High amylose corn starch + black bran extract	**22.9b**	**25.8b**	**26.2b**	**26.2b**	**26.2b**	**26.2b**	**26.2b**	**0.06a**	**22.9b**	**26.2b**
High amylose corn starch +black with tannin bran extract	**20.1b**	**22.1b**	**22.1b**	**22.1b**	**22.1b**	**22.1b**	**22.1b**	**0.08a**	**20.1b**	**22.1b**
High amylose corn starch +tannin bran extract	**10.6c**	**10.6c**	**10.6c**	**10.6c**	**10.6c**	**10.6c**	**10.6c**	**0.43b**	**10.6c**	**10.6c**
LSD	**2.1**	**3.5**	**3.1**	**4.0**	**4.0**	**4.0**	**4.0**	**0.03**	**4.0**	**4.0**

***Values within each column with different letters are significantly different at P<0.05.**

APPENDIX C

TABLE C-I

Pasting Curves of for Double Cooked Corn Starch Porridges Cooked by Rapid Visco-analyzer



VITA

Dilek (Lemlioğlu) Austin is originally from Ankara, Turkey. She obtained both her Bachelor of Science and Master of Science degrees in Food Science & Technology and Food Engineering from Ankara University in 1991 and 1994, respectively. She also completed a Master of Science degree in Food Science & Technology at the University of Nebraska-Lincoln in 1999. She was continuously employed as a Food Engineer with the Ministry of Agriculture and Rural Affairs in Turkey from 1992 through 2003, where she worked with small grains, food processing and fisheries during this time. Most recently she has been employed as a student technician at Texas A&M University during her doctoral program in the Intercollegiate Faculty of Nutrition and Food Science, Department of Crop and Soil Sciences in College Station, Texas. Dilek is fluent in English and Conversant in Spanish and has served as a consultant for the European Union Committee within the Agricultural Ministry of Turkey as an officer in charge of international relationships of Turkish agriculture. Her research interests through her various degrees and projects include studies on the composition of fatty acids and β-position fatty acids in some varieties of rapeseed-oils, identifying the impact of water absorption, mixing time, and cooking time on starch characteristics of pasta, and identifying the effects sorghum, *Sorghum bicolor* (L.) Moench, phenolic compounds on starch digestibility of porridges. Dilek is a member of the International Food Technologists (IFT), American Association of Cereal Chemists (AACC), Phi Kappa Phi Honor Society and a Regent Fellow (2005-2006) of Texas A&M University, College of Agriculture and Life Sciences. She has won numerous scholarships, graduate awards and travel grants and actively participates in meetings at both the national and international level. Her contact address is: Department of Soil & Crop Sciences, Texas A&M University, College Station, TX 77843. Her E-mail address jamesdilek1998@yahoo.com.