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A Novel Nutraceutical Property of Select Sorghum (*Sorghum bicolor*) Brans: Inhibition of Protein Glycation

Johnetta L. Farrar[1], Diane K. Hartle[1], James L. Hargrove[2] and Phillip Greenspan[1]*

[1]Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Nutraceutical Research Laboratories, Athens, GA 30602, USA
[2]Department of Foods and Nutrition, University of Georgia, Nutraceutical Research Laboratories, Athens, GA 30602, USA

Despite the high levels of polyphenolic phytochemicals in grain sorghum and its position as a major food staple, there has been a lack of research on its effects on both animal and human health and disease prevention. These phenolic compounds, mainly located in the bran fraction, result in the plant having substantial antioxidant properties. This study examined the effect of ethanol extracts of several varieties of sorghum (*S. bicolor*) bran on albumin glycation, a non-enzymatic process thought to be important in the pathogenesis of many diabetic complications. Sorghum brans with a high phenolic content and high antioxidant properties inhibited protein glycation, whereas sorghum brans that are low in these properties did not inhibit this process. Ethanol extracts of wheat, rice or oat bran did not inhibit protein glycation. Although one high phenolic sorghum bran variety (sumac) inhibited protein glycation by approximately 60%, it produced only a 20% decrease in methylglyoxal mediated albumin glycation. These results suggest that certain varieties of sorghum bran may affect critical biological processes that are important in diabetes and insulin resistance. These results distinguish select sorghum brans from the common food brans and suggest a nutraceutical rationale for its human consumption. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: sorghum bran; diabetes mellitus; protein glycation; advanced glycation endproducts (AGEs); albumin glycation.

INTRODUCTION

The consistent consumption of foods that contain significant levels of phytochemicals and dietary fiber correlates with tangible disease prevention. For example, whole grain consumption is known to help in reducing the incidence of heart disease, diabetes and other chronic diseases partly due to components in cereal brans, especially dietary fiber and phytochemicals (Awika *et al.*, 2005; Kushi *et al.*, 1999). This has led to United States dietary guidelines that recommend the increased consumption of whole grains.

Sorghum grain has been a dietary staple for millennia in parts of India, Africa and China (O'Kennedy *et al.*, 2006). Much of the growth in the world's population is in the semiarid, developing countries where drought-tolerant sorghum and millet varieties are major crops (Awika and Rooney, 2004). Some sorghum varieties have extremely high contents of phenolic compounds that aid in the natural defense of plants against pests and diseases. These phenolic compounds, mainly located in the bran fraction, result in the plant having significant antioxidant properties (Awika and Rooney, 2004). Sorghum phenolic compounds fall into two major categories: phenolic acids and flavonoids. The phenolic acids are benzoic or cinnamic acid derivatives, whereas the flavonoids are largely tannins and anthocyanins (Awika and Rooney, 2004; Dykes and Rooney, 2006).

Despite the high levels of phytochemicals in sorghum and its position as a major grain, there has been a paucity of medical research on its effects on human health. The effect of sorghum on pathological processes, such as inflammation, has not been examined. This communication tests the hypothesis that ethanol extracts of sorghum brans will inhibit protein glycation. This non-enzymatic reaction between reducing sugars and proteins is inhibited by antioxidants such as flavonoids (Wu and Yen, 2005) and is thought to be extremely important in the pathogenesis of diabetic complications. The results presented suggest that ingestion of sorghum bran could have previously unrecognized health benefits especially important to metabolic syndrome and diabetes patients.

MATERIALS AND METHODS

Chemicals. Bovine serum albumin (BSA) (Fraction V, Essentially Fatty Acid Free, D-(-)fructose, Chelex 100 (sodium form), Folin-Ciocalteu reagent, methylglyoxal solution, and TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) were purchased from Sigma Chemical Company (St Louis, MO). Sorghum brans were gifts from Dr Lloyd Rooney of Texas A & M University and Dr Scott Bean, USDA, Manhattan, KS. Rice and wheat brans were purchased from Bob's Red Mill Natural Foods, Inc (Milwaukee, OR). Oat bran was obtained from the bulk food section of a health food store in Athens, Georgia.

* Correspondence to: Dr Phillip Greenspan, University of Georgia, College of Pharmacy, Athens, GA 30602, USA.
E-mail: Greenspn@rx.uga.edu
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Preparation of sorghum extracts. To prepare the bran extracts, dried bran was made into a powder with a commercial coffee grinder. The bran was extracted with 50% ethanol (10% w/v) for 2 h at room temperature with periodic vortexing. The extract was then centrifuged to remove the precipitate and filter sterilized to obtain the final extract.

Total phenolic content. Phenolic content of samples was determined by the Folin-Ciocalteu method as described by Slinkard and Singleton (1977). Gallic acid was employed as the standard. Absorbance was measured at 765 nm in a Beckman DU 600 series spectrophotometer. The results are expressed as mg gallic acid equivalents/g bran.

Ferric reducing antioxidant power (FRAP) values. FRAP values were determined by a modified version of the method of Benzie and Strain (1996), with ferrous sulfate as the reference standard. Absorption was measured at 593 nm in a Beckman DU 600 series spectrophotometer. The FRAP assay is based on the reduction of a ferric 2,4,6-tripyridyl-S-triazine complex to the ferrous form. The results are expressed as mmol ferrous sulfate formed/100 g dry weight of bran.

Albumin glycation. The fluorescence assay to determine the glycation of albumin was performed as described by McPherson *et al.* (1988) and modified by Kim and Kim (2003). Bovine serum albumin (BSA; 10 mg/mL) was incubated with D-(-)fructose (250 mM) in 200 mM potassium phosphate buffer (pH 7.4) containing 0.02% sodium azide in a 5% CO_2 incubator at 37 °C for 72 h. The buffer was treated with Chelex 100 prior to use. Various concentrations of the extracts were added to the 3 mL incubation mixture. To control for the ethanol present in the extract, control mixtures were incubated in the presence of the appropriate concentration of ethanol. The fluorescence intensity was measured at an excitation/emission wavelength pair of 370/440 nm using a Perkin-Elmer LS 55 luminescence spectrometer. Slit widths were set at 3 nm. Values were corrected for the intrinsic fluorescence of bran extracts.

Modification of albumin by methylglyoxal. Bovine serum albumin (100 μM) was incubated with 1 mM methylglyoxal in 0.1 M sodium phosphate, pH 7.0 (Vander Jagt *et al.*, 1992). The buffer was treated with Chelex 100 prior to use. After 96 h, the fluorescence was measured using the wavelength pair of 350 and 409 nm. Values were corrected for the intrinsic fluorescence of bran extracts.

Statistical analysis. Experiments were performed in triplicate. Values are expressed as mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) and multiple comparisons were performed employing the Duncan's multiple range test. Statistical significance was set at $p < 0.05$.

RESULTS

Sorghum bran (sumac variety) was extracted with 50% ethanol (10% w/v) and the phenolic content of the extract was determined. Sumac bran had a phenolic content of 62.4 mg/g of dry weight and a FRAP value of 28.4 mmol/100 g of dry weight, in agreement with the high antioxidant capacity of this tannin-rich sorghum (Awika *et al.*, 2005). The effect of sumac sorghum bran extract on the glycation of albumin was initially examined at four different dilutions of the extract (Fig. 1). Control incubations of fructose and albumin resulted in significant albumin glycation; the relative fluorescence intensity was found to be approximately 135 units. When fructose and albumin were incubated in the presence 1:300 and 1:600 dilutions of the sorghum extract, a significant concentration dependent decrease in fluorescence intensity was observed. The 1:300 dilution produced a 60% inhibition of protein glycation. Aminoguanidine, a known inhibitor of protein glycation, decreased protein glycation by 95%. When the 1:1200 and 1:2400 dilutions were examined, a significant difference from the control incubation was not observed.

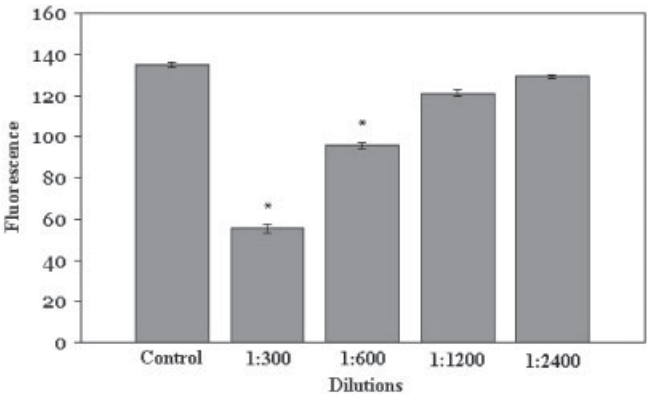

Figure 1. Effect of sumac sorghum bran extract on albumin glycation. Bovine serum albumin (10 mg/mL) was incubated with fructose (250 mM) in potassium phosphate buffer (200 mM, pH 7.4) and treated with varying concentrations of the extract for 72 h at 37 °C. The fluorescence intensity was measured at an excitation/emission wavelength pair of 370/440 nm. Results represent the mean ± SEM of triplicate determinations. * $p <$ 0.05 when compared with controls.

Since sumac sorghum bran significantly inhibited protein glycation, five other sorghum brans were examined to determine their effect on this non-enzymatic reaction. Ethanol extracts of four sorghum brans, sumac, shanqui, black and mycogen varieties at dilutions of 1:300 significantly inhibited the glycation of albumin. Fontanelle and white sorghum bran varieties, however, did not produce a significant decrease in fluorescence intensity at this dilution (Fig. 2). The phenolic content and the FRAP values for the extracts of these sorghum brans were also determined (Table 1). Fontanelle and white were found to have a very low content of phenolic compounds and low FRAP values, while the sorghum brans that inhibited glycation (sumac, shanqui, black and mycogen) had much greater phenolic contents and antioxidant FRAP values. The high level of antioxidants present in sumac bran and black bran and relatively low level present in white bran are in agreement with previous reports (Awika *et al.*, 2003, 2005).

Since certain varieties of sorghum brans were found to significantly inhibit albumin glycation, the effect of other cereal brans was also examined. As seen in Fig. 3, the glycation of albumin was not significantly inhibited by rice, wheat, or oat bran. The phenolic content and FRAP values of the bran extracts were

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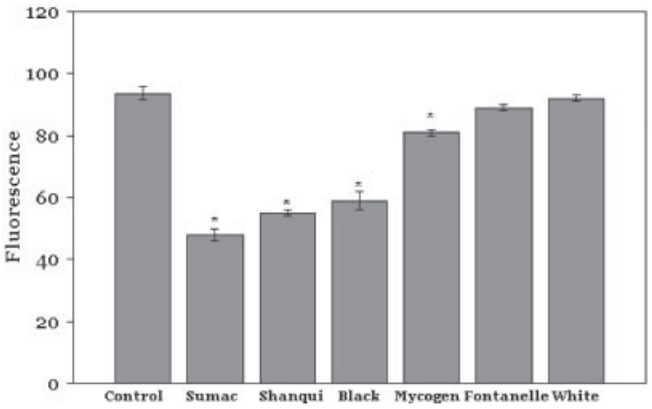

Figure 2. Effect of various sorghum bran extracts on albumin glycation. Bovine serum albumin (10 mg/mL) was incubated with fructose (250 mM) in potassium phosphate buffer (200 mM, pH 7.4) and treated with a 1:300 dilution of various extracts for 72 h at 37 °C. The fluorescence intensity was measured at an excitation/emission wavelength pair of 370/440 nm. Results represent the mean ± SEM of triplicate determinations. * $p <$ 0.05 when compared with controls.

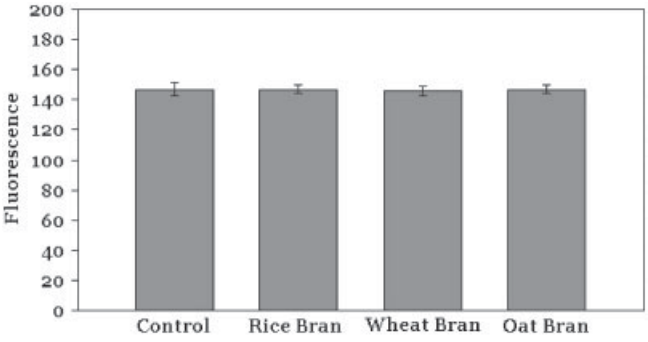

Figure 3. Effect of various bran extracts on albumin glycation. Bovine serum albumin (10 mg/mL) was incubated with fructose (250 mM) in potassium phosphate buffer (200 mM, pH 7.4) and treated with a 1:300 dilution of the various bran extracts for 72 h at 37 °C. The fluorescence intensity was measured at an excitation/emission wavelength pair of 370/440 nm. Results represent the mean ± SEM of triplicate determinations.

Table 1. Phenolic content and FRAP values of various sorghum bran and cereal bran extracts

Extract	Phenolic value (mg/g)	FRAP value (mmol/100 g)
Sorghum brans		
Sumac Sorghum bran	62.4 ± 0.9	28.4 ± 0.1
Shanqui Sorghum bran	47.7 ± 0.7	15.3 ± 0.1
Black Sorghum bran	22.7 ± 0.5	7.9 ± 0.2
Mycogen Sorghum bran	5.6 ± 0.1	2.2 ± 0.1
Fontanelle Sorghum bran	2.5 ± 0.1	1.0 ± 0.1
White Sorghum bran	3.9 ± 0.1	1.2 ± 0.1
Non-Sorghum brans		
Wheat bran	2.0 ± 0.1	0.7 ± 0.1
Oat bran	0.6 ± 0.1	0.4 ± 0.1
Rice bran	6.0 ± 0.3	5.3 ± 0.6

Data represent mean ± SEM of triplicate determinations.

examined (Table 1). In contrast to the high phenolic content and FRAP values in certain sorghum varieties, these non-sorghum brans possessed a very low content of phenolic compounds and were found to have anti-

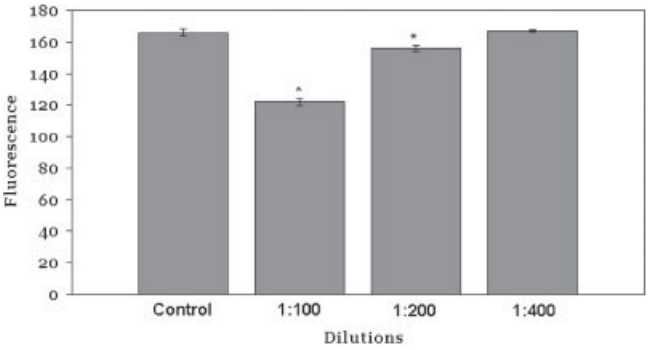

Figure 4. Effect of sumac sorghum bran on methylglyoxal induced albumin fluorescence. Bovine serum albumin (100 μM) was incubated with 1 mM methylglyoxal in 0.1 M sodium phosphate, pH 7.0 and treated with varying concentrations of sumac sorghum bran at 37 °C. After 96 h, the fluorescence was measured using the wavelength pair of 350 and 409 nm. Results represent the mean ± SEM of triplicate determinations. * $p <$ 0.05 when compared with control incubations.

oxidant capacities comparable to the low phenolic sorghum varieties.

Methylglyoxal has been shown to be an important intermediate in the autooxidation of reducing sugars and can readily glycate proteins (Lee *et al.*, 1998). Protein glycation by methylglyoxal is a nonenzymatic modification whereby arginine and lysine side chains of proteins participate in forming a heterogeneous group of advanced glycation end-products (Wu and Monnier, 2003). As seen in Fig. 4, methylglyoxal, when incubated with albumin produced significant fluorescence at the wavelength pair of 350 and 409 nm, indicative of albumin glycation. When two dilutions (1:100 and 1:200) of the ethanol extract of sumac sorghum bran were incubated with methylglyoxal, a significant decrease in fluorescence intensity was observed. The 20% inhibition of methylglyoxal induced glycation produced by sumac sorghum bran at a dilution of 1:100 was much less than the inhibitory effect observed for fructose-mediated glycation at a dilution of 1:300 (Fig. 1). A dilution of 1:400 of the sumac sorghum bran did not produce a significant decrease in methylglyoxal mediated glycation.

DISCUSSION

The glycation of proteins by reducing sugars is mediated by several processes (Baynes and Thorpe, 1999). Wolff and Dean (1987) demonstrated the role of metal-ion mediated oxidation of glucose in the glycation process while other investigators have pointed to the role of reactive carbonyl intermediates such as methylglyoxal in this protein modification (Vander Jagt *et al.*, 1992). Antioxidants such as vitamin E (Ceriello *et al.*, 1988) and flavonoids (Yokozawa and Nakagawa, 2004; Cervantes-Laurean *et al.*, 2006; Wu and Yen, 2005) have illustrated the importance of oxidation in the glycation of proteins by reducing sugars. Aminoguanidine, the classic inhibitor of protein glycation, is both an antioxidant (Giardino *et al.*, 1998) and is capable of trapping reactive carbonyl compounds (Liggins and Furth, 1997).

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The data presented here support the hypothesis that phenolic compounds in certain sorghum bran varieties significantly inhibit protein glycation mediated by the reducing sugar fructose. The inhibitory sorghum brans were those with high phenolic content and high antioxidant FRAP values. Experimental manipulation has led to the development of sorghum brans that are either high in tannins (sumac) or very low in these compounds (white, fontanelle). Sumac and black sorghum bran varieties contain anthocyanins such as luteolinidin and apigeninidin (Dykes *et al.*, 2005) and are the high phenolic varieties that strongly inhibit the glycation reaction. These results are in agreement with previous findings that demonstrated that plant extracts with high contents of flavonoids (Kim and Kim, 2003) can significantly inhibit protein glycation.

In comparison with sorghum, other cereal brans examined, such as oat, rice and wheat, had low phenolic contents and low antioxidant FRAP values. These findings on the antioxidant properties of the brans are in agreement with the findings of Awika *et al.* (2003). Sumac sorghum bran was found to have 25 times higher antioxidant capacity than the red wheat bran as measured by the antioxidant ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay (Awika *et al.*, 2005). Using another antioxidant assay, the TEAC (trolox equivalent antioxidant capacity) assay, wheat bran was shown to have three times the antioxidant capacity than oat bran (Martinez-Tome *et al.*, 2004). Therefore, the antioxidant properties observed here are consistent with those measured by other investigators.

Although sumac sorghum bran was a potent inhibitor of fructose mediated protein glycation, its effect on methylglyoxal-induced glycation was not as great. For instance at a dilution of 1:200, an extract of sorghum bran sumac produced an 8% inhibition in methylglyoxal mediated albumin glycation, whereas a 1:300 dilution produced a 60% reduction in fructose mediated glycation. The failure to block the methylglyoxal reaction suggests that oxidative processes may not participate in the glycation of albumin mediated by methylglyoxal to same extent as it does in fructose-induced glycation. A similar profile of strong inhibition of glucose mediated glycation and weak, but significant, inhibition of methylglyoxal inhibition was observed for epicatechin and epigallocatechin (Wu and Yen, 2005).

This study illustrates that select sorghum brans have potential to modulate processes involved in inflammatory diseases. The present results show that phenolic-rich sorghum brans inhibit a non-enzymatic process thought to be important in the pathogenesis of diabetic complications, such as neuropathy and cardiovascular disease. Morimitsu *et al.* (1995) demonstrated that extracts of thyme and its constituent flavonoids inhibited protein glycation. Since that observation, extracts of herbs and spices (Ahmad and Ahmed, 2006; Bae and Lee, 2004) and plants (Kim and Kim, 2003; Nakagawa *et al.*, 2002) have demonstrated antiglycation activity. In one study, 25 different plant tissues were examined and it was found that inhibition of glycation was significantly correlated with antioxidant capacity (Kim and Kim, 2003). Recently, ten different flavonoids were tested for inhibition of glucose-mediated albumin glycation; all ten flavonoids inhibited glycation with the IC_{50} values ranging from 16 μM (luteolin) to 160 μM (epigallocatechin) (Wu and Yen, 2005).

The effect of food staples on protein glycation has not received significant attention. A water soluble, low-molecular weight extract of tomato paste inhibited protein glycation with rutin thought to be responsible for a significant portion of the inhibitory activity (Kiho *et al.*, 2004). Millets, from the Kodo and Finger varieties, have also been shown to inhibit protein glycation (Hegde *et al.*, 2002). The results of this study add specialty sorghum brans to the list of food staples that inhibit glycation and confirm that these sorghum brans are far superior in this property when compared with other brans commonly used in the diet. The high antioxidant and antiglycation properties provide further rationale for nutraceutical and food ingredient use of select sorghum brans and their extracts.

It is known that high tannin sorghums reduce caloric availability and weight gain in animals (Awika and Rooney, 2004) and this property can be potentially exploited in managing obesity and metabolic syndrome, two conditions associated with type II diabetes. This current work demonstrates yet another property of high tannin sorghum, that of potentially decelerating the rate of AGE product formation, inherent in complications observed in chronically high glucose metabolic conditions. The work provides further rationale for human clinical investigations to reintroduce sorghum phytochemicals into the modern diet for metabolic disease management. This can be accomplished via a food ingredient, food supplement or nutraceutical product.

Acknowledgements

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