JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Processing of Sorghum (*Sorghum bicolor*) and Sorghum Products Alters Procyanidin Oligomer and Polymer Distribution and Content

JOSEPH M. AWIKA,*,† LINDA DYKES,† LIWEI GU,‡ LLOYD W. ROONEY,† AND RONALD L. PRIOR‡

Cereal Quality Lab, Texas A&M University, College Station, Texas 77843-2474 and USDA, ARS, Arkansas Children's Nutrition Center, Little Rock, AR

Sorghum procyanidins were characterized and quantified from two brown sorghum varieties and their processed products by normal phase HPLC with fluorescence detection. The DP of the procyanidins was determined by thiolysis. Quantification was done by using purified oligomeric and polymeric cocoa procyanidins as external standards. Sorghum procyanidins were composed mostly of high MW (DP > 10) polymers. Significant differences were observed in levels as well as distribution of the different MW procyanidins between the sorghums. Processing of the sorghum brans into cookies and bread significantly reduced the levels of procyanidins; this effect was more pronounced in the higher MW polymers. Cookies had a higher retention of procyanidins (42−84%) than bread (13−69%). Extrusion of sorghum grain resulted in an increase in the levels of procyanidin oligomers with DP ≤ 4 and decrease in polymers with DP ≥ 6. This suggests a possible breakdown of the high MW polymers to the lower MW constituents during extrusion. Processing changes not only the content of procyanidins in sorghum products but also the relative ratio of the different molecular weights.

KEYWORDS: Sorghum; procyanidins; HPLC; extrusion; baking.

INTRODUCTION

Condensed tannins, commonly called procyanidins, (**Figure 1**) are a major phenolic component of sorghums with a pigmented testa. They are concentrated in the testa and pericarp of such sorghums. Sorghum tannins have generally been viewed as undesirable, due to their antinutritive properties (i.e., they complex with food macromolecules reducing their digestibility) (*1*−*3*). However, current data suggest that tannins may have nutraceutical benefits, due to their powerful antioxidant activity (*4*−*8*). Consequently, research efforts are directed at understanding their precise mode of action and bioavailability in biological systems and how their consumption can be enhanced in human diets. Recent data suggest that procyanidins and other flavonoids are more bioavailable than previously thought (*9*, *10*). Deprez et al. (*11*) reported that procyanidins could be absorbed through the intestinal cell monolayer, but only up to trimers. However, the interflavan bond in the procyanidins was reportedly unstable in acid (simulated gastric juice) environment, suggesting the higher molecular weight procyanidins could be broken down in the stomach (*12*), enhancing their potential bioavailability. Specialty sorghums rich in these compounds (*13*) need detailed analysis to determine how they can be effectively used to improve human health.

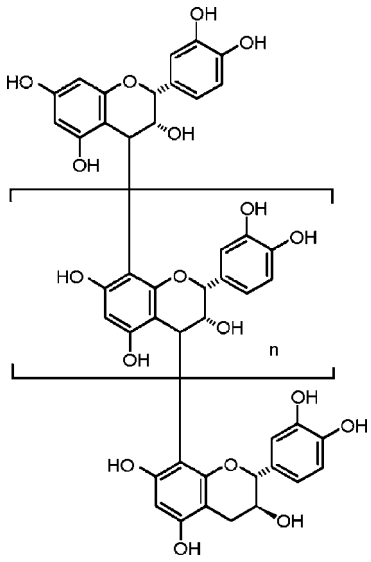

Figure 1. Structure of condensed tannin commonly found in brown sorghum.

Consumption in food may be a more effective way to deliver nutraceutical compounds such as procyanidins to consumers than through dietary supplements. This is becoming increasingly important given that the safety and efficacy of the concentrated dietary supplements has been questioned (*14*−*16*). Cereal-based

* Corresponding author. Tel.: 979-845-2925. Fax: 979-845-0456. E-mail: jawika@tamu.edu.
† Texas A&M University.
‡ Arkansas Children's Nutrition Center.

10.1021/jf0343128 CCC: $25.00
Published on Web 07/26/2003

foods are an important vehicle to deliver such compounds to consumers because they are consumed widely and consistently as staples in many parts of the world. The tannin-containing sorghums are a promising source of these compounds in such foods. It is thus important to characterize changes in their procyanidin profiles and content during food processing to better estimate their potential contribution as a source of health components in food.

Colorimetric assays (e.g., vanillin-HCl) are the most common methods used to estimate tannin content. However, structural complexity of tannins as well as lack of appropriate standards make accurate estimations of tannin contents based on such methods difficult. Additionally, such methods give no information on the relative distribution of different MW procyanidin polymers in a given sample. High performance liquid chromatography (HPLC) is recognized as an effective tool for characterizing procyanidins in natural products (*17*−*19*) and more recently as a useful tool for their quantification (*20*, *21*). Adamson et al. (*20*) separated and quantified proyanidin monomers and oligomers (up to DP 10) from cocoa and chocolate using normal-phase HPLC with fluorescence detection. Gu et al. (*21*) optimized the method of Adamson et al. (*20*) for berries, cocoa, and sorghum procyanidins. They were able to resolve the polymers with DP > 10 as a single peak that could be quantified, in addition to the monomers and DP 2−DP 10 oligomers. They found the high molecular weight polymers were the major procyanidins in brown sorghum bran, cocoa, cranberries, and blueberries.

HPLC presents a more powerful tool for quantification of procyanidins in sorghums than spectrophotometry methods, due to its specificity and ability to detect contribution of different MW procyanidins to the total procyanidin content of a sample. This is important in health and food applications because polymer chain length affects organoleptic, antioxidant, and other potential health properties of procyanidins (*18*, *22*, *23*).

The objectives of this study were to use the newly available improved normal-phase HPLC coupled with fluorescence detection to evaluate procyanidin profiles and contents of selected brown sorghums and obtain preliminary information on the effect of processing on the procyanidin profiles of these sorghums.

MATERIALS AND METHODS

Samples. Two brown sorghum varieties were used, Sumac grown in Vega, TX in 1999 and Hi Tannin grown in College Station, TX in 2001. The samples were decorticated using a PRL dehuller (Nutama Machine Co., Saskatoon, Canada) to obtain bran fractions. Bran yields were 12% for each sample. Bread samples made by Gordon (*24*) containing 30% Sumac bran (flour basis) and cookies made by Mitre-Dieste (*25*), containing 50% of the Sumac bran (flour basis) were also analyzed. The bread and cookie samples were kept at < −20 °C in the dark in plastic moisture proof containers until analyzed. In addition, the Hi Tannin grain was extruded whole through a friction-type Maddox single screw extruder, model MX-3001 (Maddox Metal Works, Inc., Dallas, TX). Screw speed was 300 rpm, die diameter was 6.125 in., and sample moisture was 12% (non tempered).

Standards. (−)-Epicatechin and (±)-catechin were purchased from Sigma Chemical Co. (St Louis, MO). Procyanidin oligomers (DP 2−DP 10) were purified from cocoa as detailed by Hammerstone et al. (*19*) and Adamson et al. (*20*). A mixture of purified polymers as standard for >DP 10 procyanidins.

Sample Extraction. All extraction solvents were HPLC grade. Samples were ground through a UDY mill (1 mm mesh) prior to extraction. The bread and cookie samples were initially dried in a forced-air convection oven at 50 °C for 8 h before grinding.

The method described by Gu et al. (*21*) was used for extraction. In brief, 1 g samples were extracted in 10 mL of acetone/water/acetic acid (70:29.5:0.5). Samples were sonicated at 37 °C for 10 min, let stand at room temp for 50 min, and then centrifuged at 3500 rpm for 15 min. Acetone was evaporated from the extract at 25 °C in a SpeedVac (SC201A, Thermo, Marietta, OH) under vacuum, and the residues were dissolved in 6 mL of water and applied to a Sephadex LH-20 column. The column was washed with 40 mL of 30% methanol in water to remove sugars and other phenols, and the procyanidins were recovered with 80 mL of aqueous acetone (70% v/v). The eluents were evaporated to dryness under vacuum at 43 °C in a SpeedVac, redissolved in 5 mL of the extraction solvent, and filtered (0.45 μm) before HPLC analysis. Recovery rates for the procyanidins were determined using the purified procyanidin standards as described by Gu et al. (*21*). The rates were used to adjust measured procyanidin fraction contents.

Determination of DP of Procyanidins. This was performed by thiolysis as detailed by Gu et al. (*21*). Each procyanidin fraction separated as detailed by Adamson et al. (*20*) was extracted with acidified methanol (3.3% HCl v/v) and benzyl mercaptan (5% v/v in methanol) for 30 min at 40 °C and kept at room temp for 10 h to ensure complete degradation. The reaction mixture was then stored at −18 °C before analysis. An Agilent 1100 HPLC system coupled with a Bruker Esquire-LC ion trap mass spectrometer was used for analysis. The components in thiolysis media were identified with the mass spectrometer in negative mode using a setting of 50% for compound stability and 80% for ion trap drive level. Detail on the analysis of thiolysis products is previously described (*21*).

HPLC Analysis. The method detailed by Gu et al. (*21*) was used. Mobile phase was (A) dichloromethane, (B) methanol, and (C) acetic acid/water (1:1 v/v). Gradient was 0−30 min, 14.0−28.4% B; 30−45 min, 28.4−39.6% B; 45−50 min, 39.6−86.0% B; 50−55 min, 86.0% B isocratic; 55−60 min, 86.0−14.0% B; followed by 10 min reequilibration of the column before the next run. A constant 4% C was maintained throughout the gradient. Flow rate was 1 mL/min. Separation was on a normal-phase 5-μL Luna silica column (250 × 46 mm) (Phenomenex, Torrance, CA). Fluorescence detection was used; excitation − 276 nm, emission − 316 nm.

RESULTS AND DISCUSSION

Grain and Bran Samples. Figure 2 shows the HPLC procyanidin profiles of the two sorghum grains. The method was able to resolve procyanidins up to decamers based on molecular weight. Additionally, the polymers with DP > 10 were resolved as a single peak, as previously demonstrated by Gu et al. (*21*). The two sorghum grains analyzed showed significant differences not only in their levels of procyanidins but also in the distribution of the individual oligomers and polymers. The Hi Tannin grain had a smaller ratio of the lower molecular weight oligomers (DP < 10) to the polymers (DP > 10) than the Sumac grain (**Figure 2**, **Table 1**). The Hi Tannin sorghum had only 14% oligomers as a proportion of the total procyanidins, compared to the Sumac grain, which had 31% oligomers. Because procyanidin chain length is reported to affect their effectiveness against different oxidative stresses (*22*, *23*), information on relative proportions of the different procyanidin oligomers as well as polymers may be useful in predicting overall effectiveness of sorghum procyanidins as antioxidants in vivo. A good mix of oligomers and polymers is essential for better overall antioxidant protection (*23*). The Sumac bran, as expected, had a significantly higher level of procyanidins than the grain. Sorghum procyanidins are found in the pericarp and testa, and decortication concentrates their levels 3−7-fold (*13*, *26*). Sorghum decortication is an effective way of concentrating sorghum procyanidins for potential commercial exploitation.

Comparison of procyanidin oligomer and polymer distribution and contents of sorghum grains and bran with literature values

Table 1. Procyanidin Content[a] of Brown Sorghums Compared to Those of Freeze-Dried Cocoa and Blueberry[g]

DP[b]	hi tannin sorghum grain	sumac sorghum grain	sumac sorghum bran	cocoa[c]	blueberry[c]
1	0.01 ± 0.00	0.18 ± 0.01	0.33 ± 0.07	14.24 ± 0.38	0.18 ± 0.01
2	0.09 ± 0.01	0.40 ± 0.01	1.33 ± 0.26	8.57 ± 0.51	0.46 ± 0.02
3	0.12 ± 0.01	0.51 ± 0.01	1.61 ± 0.33	8.10 ± 0.49	0.38 ± 0.02
4	0.21 ± 0.03	0.69 ± 0.01	2.32 ± 0.46	8.89 ± 0.54	0.50 ± 0.01
5	0.26 ± 0.04	0.74 ± 0.01	2.51 ± 0.51	8.86 ± 0.52	0.47 ± 0.01
6	0.49 ± 0.07	1.10 ± 0.02	3.61 ± 0.71	9.99 ± 0.61	0.69 ± 0.05
7	0.38 ± 0.06	0.79 ± 0.01	2.56 ± 0.50	6.38 ± 0.38	0.48 ± 0.02
8	0.38 ± 0.06	0.74 ± 0.01	2.29 ± 0.43	5.97 ± 0.31	0.61 ± 0.03
9	0.63 ± 0.10	1.17 ± 0.02	3.48 ± 0.62	7.36 ± 0.58	0.93 ± 0.02
10	0.31 ± 0.04	0.55 ± 0.01	1.52 ± 0.26	3.22 ± 0.23	UI[e]
P[d]	17.67 ± 3.92	15.09 ± 0.34	36.87 ± 6.12	16.17 ± 0.80	15.28 ± 0.51
total	20.50 ± 4.35	21.97 ± 0.45	58.44 ± 10.27	97.76 ± 5.32	19.99 ± 0.43
% oligo[f]	14.03	31.31	36.33	83.45	23.51

[a] mg/g, obtained by normal phase HPLC with fluorescence detection. [b] Degree of polymerization. [c] Gu et al. (2002). [d] Mixture of polymers with DP > 10. [e] UI = unidentified. [f] Oligomers (DP < 10) as a percent of total. [g] Values are means ± standard deviation from two separate extractions.

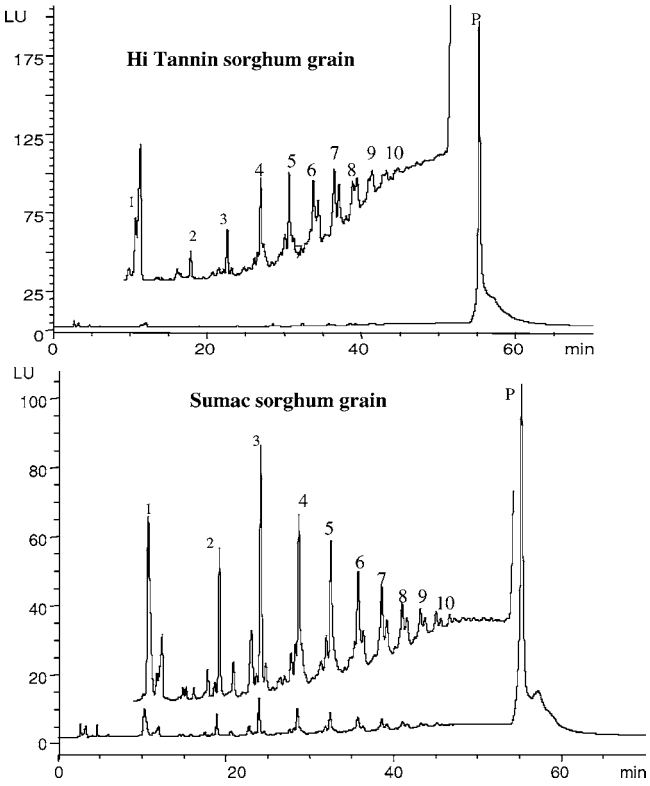

Figure 2. Normal phase HPLC procyanidin profiles of two brown sorghum grains, Sumac and Hi Tannin. Numbers on peaks denote degree of polymerization. P = mixed polymers (DP > 10). Figure insets are vertically magnified oligomer profiles.

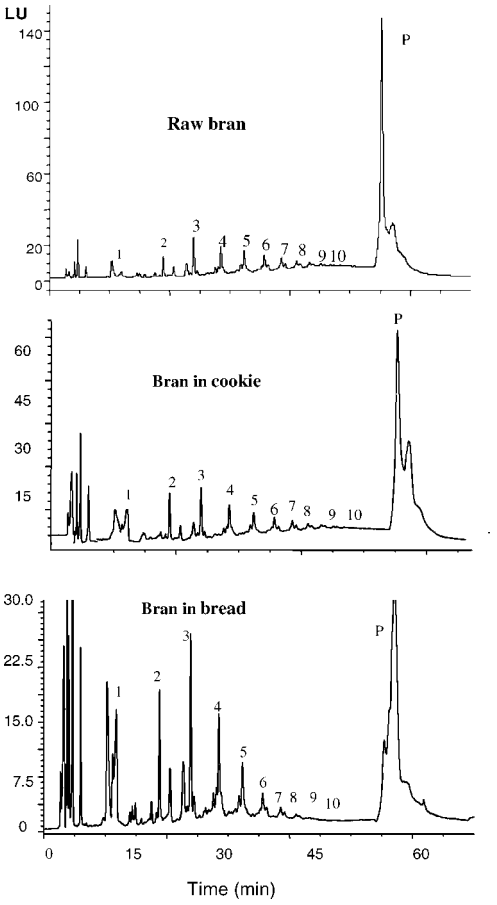

Figure 3. Normal phase HPLC procyanidin profiles of Sumac bran before and after baking in cookies and bread. Scales for cookie and bread brans are adjusted to 100% bran. Numbers on peaks denote degree of polymerization. P = mixed polymers with DP > 10.

for blueberry and cocoa are shown in **Table 1**. The Sumac grain and bran had higher levels of procyanidins and a higher ratio of oligomers relative to polymers than blueberry. This sorghum variety could thus be a good source of the procyanidins for nutritional or health applications. The Hi Tannin grain had procyanidin content similar to that of blueberry, but blueberry had a higher ratio of oligomers to polymers. Cocoa had the highest level of procyanidins and the highest ratio of oligomers to polymers. The procyanidins were previously reported as the primary contributors of antioxidant activity in cocoa (*20*).

Baked Products. The procyanidins in brown sorghum are known to bind food macromolecules, especially proteins (*27*, *28*) and carbohydrates (*29*), forming insoluble complexes. Such complexes are harder to extract for analysis. Because the higher molecular weight (MW) procyanidins are the ones mostly involved in these interactions (*30*−*32*), it was expected that their extractability (and hence measured levels) would decrease more significantly compared to the lower MW procyanidins in the baked products. This was observed in the HPLC profiles of the processed Sumac bran tannins relative to the unprocessed brans (**Figure 3**). The relative peak heights and quantities of the lower molecular weight tannins compared to higher molecular weight tannins were higher in the processed brans than in raw brans (**Figure 3**, **Table 2**). This effect was more pronounced in bread than in the cookies, as is clearly visible in **Figure 3**.

Table 2. Effect of Processing on Procyanidin Polymer Contents of Sumac Sorghum Bran Measured by Normal-Phase HPLC

	raw bran		bran in cookies			bran in bread		
DP[a]	content (mg/g)	dist[b] (%)	content (mg/g)	dist[b] (%)	% loss[c]	content (mg/g)	dist[b] (%)	% loss[c]
1	0.33	0.6	0.29	1.0	12.1	0.24	1.4	27.3
2	1.33	2.6	0.90	3.2	32.3	0.91	5.4	31.6
3	1.63	2.8	0.88	3.1	46.0	0.97	5.8	40.5
4	2.31	4.0	1.20	4.3	48.1	1.06	6.4	54.1
5	2.51	4.3	1.16	4.1	53.7	0.83	5.0	66.9
6	3.61	6.2	1.65	5.9	54.3	1.04	6.2	71.2
7	2.56	4.4	1.10	3.9	57.0	0.54	3.2	78.9
8	2.29	3.9	0.98	3.4	57.2	0.43	2.6	81.2
9	3.48	6.0	1.49	5.3	57.2	0.52	3.1	85.1
10	1.52	2.6	0.66	2.3	56.6	0.21	1.2	86.2
P[d]	36.87	63.1	17.77	63.3	51.8	9.90	59.4	73.1
total	58.44		28.06		52.0	16.66		71.5
CV[e]	10.2	10.2	5.5	5.5	5.5	4.1	4.1	4.1

[a] Degree of polymerization. [b] Relative distribution of the different molecular weights. [c] Reduction in level of measurable procyanidins after processing. [d] Mixture of polymers with DP > 10. [e] Coefficient of variaton (%), based on means from two separate extractions.

In the Sumac bran cookies, procyanidin monomer loss was only 12%, while the loss of decamers was 57% relative to raw bran (**Table 2**). Approximately 52% of the mixed polymers (>10 DP) were lost in the Sumac bran cookies relative to raw bran. In the Sumac bran bread, the procyanidins decreased more dramatically (27% loss of the monomers and 86% loss of the decamers). Only 28% of the mixed polymers were measured in the Sumac bran bread relative to raw bran (82% loss). These data clearly suggest that the degree of interaction of the procyanidins with food macromolecules during processing increases with the degree of polymerization of the procyanidins.

The bread allowed more opportunity for these interactions, due to its formulation and processing conditions. The bread formula contained about 64% water (baker's percent, i.e., based on flour weight) added (*24*). It was made by a straight dough method that involved significant amounts of mixing (12−15 min) and other prolonged (approximately 3 h) handling operations before baking. The cookie formulation, on the other hand, involved less moisture (10% water added) and significantly more sugar and fat compared to bread formulations (*24*, *25*). Mixing and other procedures preceding baking, as well as the baking itself, also involve less time for cookies than bread. Hence, the availability of moisture as a solvent for the sorghum bran procyanidins and other food molecules, as well as the degree of procyanidin interaction with other dough components, were greatly limited in cookie dough compared to bread dough. These factors ensured that the procyanidin constituents in the cookie dough bran were better retained in their original forms than in bread doughs. The baking time for the bread was also longer (approx 25 min at 420 °C) than for cookies (14 min at 375 °C). This may have resulted in more heat-induced changes to the bread bran procyanidins than the cookie bran procyanidins.

Extrusion. The HPLC profile for the high tannin sorghum and its extrudate are shown in **Figure 4**. As observed for the baked products, significant increase in peak heights for lower DP procyanidins relative to the high DP procyanidins was observed. However, the effect was more pronounced in the extrudate than in the baked products. There was a very significant increase in the levels of DP1−DP4 procyanidins, with the extractable monomers increasing by 478% and the tetramers by 29% of levels in original grain (**Table 3**). Above DP5, however, significant reduction in procyanidin levels were

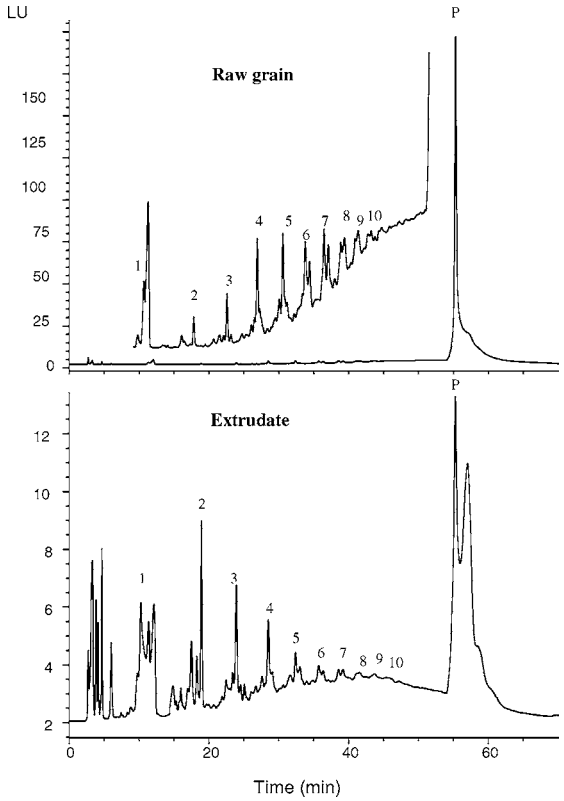

Figure 4. Normal-phase HPLC procyanidin profiles of Hi Tannin grain before and after extrusion. Grain was extruded through a friction-type single screw extruder (Maddox Metal Works, Dallas, TX) at MC of 12%. Numbers on peaks denote degree of polymerization. P = polymers with DP > 10. Inset for "raw grain" figure is a vertically magnified oligomer profile.

Table 3. Effect of Extrusion on Procyanidin Polymer Content and Distribution in Hi Tannin Sorghum Grain Measured by Normal-Phase HPLC

	raw grain		extruded grain[b]		
DP[a]	content (mg/g)	dist[c] (%)	content (mg/g)	dist[c] (%)	percent loss[d]
1	0.01	0.6	0.08	1.70	(–478.04)
2	0.09	2.6	0.25	5.24	(–180.80)
3	0.12	2.8	0.23	4.74	(–97.20)
4	0.21	4.0	0.27	5.53	(–29.22)
5	0.26	4.3	0.26	5.29	2.60
6	0.49	6.2	0.35	7.20	28.51
7	0.38	4.4	0.23	4.66	39.91
8	0.38	3.9	0.19	3.96	49.33
9	0.63	6.0	0.29	5.88	54.52
10	0.31	2.6	0.12	2.52	60.38
P[e]	17.66	63.1	2.59	53.30	85.33
total	20.51		4.86		76.30
CV[f]	10.2	10.2	0.45	0.45	0.45

[a] Degree of polymerization. [b] Whole grain extruded through a single-screw, friction-type, short-barrel Maddox MX-3001. [c] Relative distribution of the different molecular weights. [d] Reduction in level of measurable procyanidins after processing. Values in parentheses denote increased levels after processing. [e] Mixture of polymers with DP > 10. [f] Coefficient of variaton (%), based on means from two separate extractions.

observed, with the polymers (>DP10) in the extrudates recovered at only 15% of the original grain (85% loss). As observed earlier, this trend could be due to tannin polymer complexation with other sorghum constituents during extrusion. However, due to the high levels of lower DP procyanidins recovered in

extrudate relative to grain, cleavage of the higher DP procyanidins into lower DP constituents during extrusion is likely. This suggests that extrusion may help break down the high molecular weight procyanidins into their lower molecular weight constituents that are potentially easier to absorb by humans (*11*). This may, in effect, improve the nutraceutical value of brown sorghums.

In general, it is apparent that processing conditions affect overall distribution of procyanidin polymer units and their content in food. As more information becomes available on bioavailability, metabolism, and efficacy of these compounds, it will probably be possible in future to determine an optimally desirable MW distribution and levels of procyanidins in food. Then, processing conditions may be manipulated to achieve this composition.

It would be interesting to determine a complete nutrient profile of the sorghum-based baked products and extrudates to estimate how the sorghums' procyanidins affect nutrient availability. On the basis of the known tannin interactions with food macronutrients (*27*−*29*) such tannin−sorghum-based products may provide a lower calorie value than similar products without these sorghum components. Cousins et al. (*33*), and Rooney and Pflugfelder (*34*) reported significantly reduced calorie value of tannin sorghums used as feed compared to nontannin sorghums and corn. Bread, cookies, and cereal-based snack foods consumption contribute a major portion of calories in the developed world, where obesity is a growing problem. Tannin-rich sorghums and their fractions are a potentially useful means to reduce the calorie intake from such foods, besides providing other functional benefits.

For the first time, the effect of different processing conditions on contents of individual sorghum procyanidin polymer units is reported. Levels of sorghum procyanidins after processing depended on their molecular weight, with the highest retention observed in the lower molecular weight procyanidins. In the case of extrusion, an increase in levels of monomers to tetramers was observed, suggesting a breakdown of the higher molecular weight polymers to their lower molecular weight constituents. The data demonstrates that sorghum and sorghum fractions rich in procyanidins can be processed into various cereal-based foods and retain significant levels of these compounds that may have functional properties. A more detailed study is necessary to confirm the behavior of sorghum procyanidins from various sources when subjected to different processing conditions. We are also in the process of investigating how the different processing conditions as well as changes in procyanidin profiles of the sorghums affect their response to antioxidant stresses in different systems.

ABBREVIATIONS USED

HPLC, high performance liquid chromatography; MW, molecular weight; DP, degree of polymerization

LITERATURE CITED

(1) Price, M. L.; Butler, L. G. Tannins and nutrition. *Purdue Univ. Agric. Exp. Stn. Bull. No. 282*: Lafayette, IN. **1980**.

(2) Salunkhe, D. K.; Jadhav, S. J.; Kadam, S. S.; Chavan, J. K. Chemical, biochemical, and biological significance of polyphenolics in cereals and legumes. *CRC Crit. Rev. Food Sci. Nutr*. **1982**, *17*, 277.

(3) King-Thom, C.; Tit, Y. W.; Cheng, I. W.; Yao-Wen, H.; Yuan, L. Tannins and human health: a review. *Crit. Rev. Food Sci. Nutr*. **1998**, *38*, 421−464.

(4) Kinsella, J.; Frankel, E.; German, B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol*. **1993**, *47* (4), 85−89.

(5) Clifford, E. Delayed tumor onset in transgenic mice fed on amino acid-based diet supplemented with red wine solids. *Am. J. Clinical Nutr*. **1996**, *64*, 748−756.

(6) Hagerman, A. E.; Riedl, K. M.; Jones, G. A.; Sovik, K. N.; Ritchard, N. T.; Hartzfeld, P. W.; Riechel, T. K. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem*. **1998**, *46*, 1887−1892.

(7) Bors, W.; Michel, C.; Stettmaier, K. Electron paramagnetic resonance studies of radical species of proanthocyanidins and gallate esters. *Arch. Biochem. Biophys*. **2000**, *374*, 347−355.

(8) Heinennonen, I. M.; Meyer, A. S.; Frankel, E. N. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *J. Agric. Food Chem*. **1998**, *46*, 4107−4112.

(9) Pietta, P. G. Flavonoids as antioxidants. *J. Nat. Prod*. **2000**, *63*, 1035−1042.

(10) Ross, J. A.; Kasum, C. M. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu. Rev. Nutr*. **2002**, *22*, 19−22.

(11) Deprez, S.; Mila, I.; Huneau, J.; Tome, D.; Scalbert, A. Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxid. Redox Signaling* **2001**, *3*, 957−967.

(12) Spencer, J.; Chaudry, F.; Pannala, A.; Srai, S.; l Debnam, E.; Rice-Evans, C. Decomposition of cocoa procyanidins in the gastric milieu. *Biochem. Biophys. Res. Comm*. **2000**, *272*, 236−241.

(13) Awika, J. M. M. S. thesis, Texas A&M University, College Station, TX. 2000.

(14) Kim, I.; Williamson, D. F.; Bvyers, T.; Koplan, J. P. Vitamins and mineral supplements use and mortality in a US cohort. *Am. J. Public Health* **1993**, *83*, 546−550.

(15) Stephens, N. Antioxidant therapy for ischemic heart disease: where do we stand? *Lancet* **1997**, *349*, 1710−1711.

(16) Halsted, C. H. Dietary supplements and functional foods: 2 sides of a coin? *Am. J. Clin. Nutr*. **2003**, *77(suppl.)*, 1001S−1007S.

(17) Putman, L. J.; Butler, L. G. Separation of high molecular weight sorghum procyanidins by high performance liquid chromatography. *J. Agric. Food Chem*. **1989**, *37*, 943−946.

(18) Rigaud, J.; Escribano-Bailon, M. T.; Prieur, C.; Souquet, J. M.; Cheynier, V. Normal-phase high-performance liquid chromatographic separation of procyanidins from cacao beans and grape seeds. *J. Chromat. A* **1993**, *654*, 255−260.

(19) Hammerstone, J. F.; Lazarus, S. A.; Mitchel, A. E.; Rucker, R.; Schmitz, H. H. Identification of procyanidins in cocoa (*Theobroma cocoa*) and chocolate using high-performance liquid chromatography/mass spectrophotometry. *J. Agric. Food Chem*. **1999**, *47*, 490−496.

(20) Adamson, G. E.; Lazarus, S. A.; Mitchell, A. E.; Prior, R. L.; Cao, G.; Jacobs, P. H.; Kremers, B. G.; Hammerstone, J. F.; Rucher, R. B.; Ritter, K. A.; Schmidtz, H. H. HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. *J. Agric. Food Chem.* **1999**, *47*, 4184−4188.

(21) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunnigham, D.; Vannozzi, S.; Prior, L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J. Agric. Food Chem*. **2002**, *50*, 4852−4860.

(22) Tebib, K.; Rouanet, J. M.; Besancon, P. Antioxidant effects of dietary polymeric grape seed tannins in tissue of rats fed a high cholesterol-vitamin E-deficient diet. *Food Chem*. **1997**, *59* (1), 135−141.

(23) Lotito, S. B.; Actis-Goretta, L.; Renart, M. L.; Caligiuri, M., Rein, D.; Schmitz, H. H.; Steinberg, F. M.; Keen, C. L.; Fraga, C. G. Influence of oligomer chain length on antioxidant activity of procyanidins. *Biochem. Biophys. Res. Comm*. **2000**, *276*, 945−951.

(24) Gordon, L. A. M. S. thesis, Texas A&M University, College Station, TX, 2001.
(25) Mitre-Dieste, C. M.; Gordon, L. A.; Awika, J.; Suhendro, E. L.; Rooney, L. W. Cookies made with sorghum brans high in phenols and catechins. *AACC Annual Meeting Abstracts*, URL http://www.aaccnet.org/meetings/2000/abstracts/a00ma203.htm.
(26) Hahn, D. H.; Rooney, L. W. Effects of genotype on tannins and phenols of sorghum. *Cereal Chem*. **1985**, *1*, 4−8.
(27) Haslam, E. Polyphenol-protein interactions. *Biochem. J*. **1974**, *139*, 285−288.
(28) Hagerman, A. E.; Butler, L. G. The specificity of proanthocyanidin-protein interactions. *J. Biol. Chem*. **1981**, *256*, 4494−4497.
(29) Naczk, M.; Shahidi, F. Nutritional implications of canola condensed tannins. *Antinutrients and Chemicals in Food: ACS Symposium Series*. **1997**, *662*, 186−208.
(30) Bacon, J. R.; Rhodes, M. J. C. Development of a competitive assay for the evaluation of the binding of human parotid salivary proteins to dietary complex phenols and tannins using a peroxidase-labeled tannin. *J. Agric. Food Chem*. **1998**, *46*, 5083−5088.
(31) Sarni-Manchado, P.; Cheynier, V.; Moutounet, M. Interactions of grape seed tannins with salivary proteins. *J. Agric. Food Chem*. **1999**, *47*, 42−47.
(32) Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds − nature, occurrence, dietary intake, and effects on nutrition and health. *J. Sci. Food Agric*. **2000**, *80*, 1097−1117.
(33) Cousins, B. W.; Tanksley, T. D.; Knabe, D. A.; Zebrowska, T. Nutrient digestibility and performance of pigs fed sorghums varying in tannin concentration. *J. Anim. Sci*. **1981**, *53*, 1524−1529.
(34) Rooney, L. W.; Pflugfelder, R. L. Factors affecting starch digestibility with special emphasis on sorghum and corn. *J. Animal Sci*. **1986**, *63*, 16.7−1632.

Received for review March 28, 2003. Revised manuscript received June 20, 2003. Accepted June 25, 2003.

JF0343128