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Sorghum Extrusion Increases Bioavailability of Catechins in Weanling Pigs

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Catechins and procyanidins are beneficial for human health; however, their bioavailability is low. The effect of food processing on catechin bioavailability from sources containing predominantly procyanidins has not been studied. The sumac sorghum mixture (50% whole grain + 50% bran) used in this study contained catechins, procyanidins dimers, and polymers at 0.08, 0.6, and 26.4 mg/g, respectively. Extrusion decreased the polymeric procyanidins by 48% to 22 mg/g while increasing catechins (50%) and dimers (64%) to 0.12 and 1.0 mg/g, respectively. Six weanling pigs (8.9 ± 1.1 kg) received a single dose by gavage of the sorghum mixture (7 g/kg$^{0.75}$), the sorghum mixture extrudate, or white sorghum (50% whole grain + 50% bran) in a randomized crossover design. Treatments were separated by a 7-day washout period. Blood was drawn at 0, 1, 2, and 4 h. Plasma catechin, 3′-*O*-methylcatechin, 4′-*O*-methylcatechin, epicatechin, 3′-*O*-methylepicatechin, and 4′-*O*-methylepicatechin peaked at 1 h and were 18, 43, 1, 0.7, 0.7, and 0.3 nmol/L for pigs receiving sorghum, respectively. Plasma levels in pigs receiving extruded sorghum were 66, 110, 2, 16, 8, and 11 nmol/L, respectively. Plasma levels of catechin, 3′-*O*-methylcatechin, and the total catechins were higher in pigs fed extruded sorghum at 1, 2, and 4 h postdose ($P \leq 0.05$). The majority of the absorbed catechins were excreted within 4 h after feeding. Urinary excretion of total catechins was significantly higher in pigs fed extruded sorghum than in those fed nonextruded sorghum. Procyanidin dimers were not detected in plasma or urine. The levels of catechins were close to zero in plasma and urine of pigs fed white sorghum. In conclusion, extrusion improved the bioavailability of catechins in sorghum.

KEYWORDS: Sorghum; procyanidins; catechin; epicatechin

INTRODUCTION

Sorghum is the fifth most important cereal crop in the world after wheat, rice, corn, and barley. More than 35% of the sorghum is grown directly for human consumption. The United States is the largest producer and exporter of sorghum, accounting for 20% of world production and almost 80% of world sorghum exports during 2001–2002 (*1*).

Different varieties of sorghum can be roughly divided into two categories: the tannin-free sorghums, such as the white sorghum; and the tannin sorghums, such as the brown sorghum. Tannins are the most unique and important constituents in tannin sorghum because of their high concentrations and various effects on human health (*2*–*4*). Tannins in sorghum are almost exclusively procyanidins (*2*). Other proanthocyanidins with greater heterogeneity have previously been identified in sorghum (*5*), but they appear to be minor components. Procyanidins in sorghum are the oligomers and polymers of catechin or epicatechin. The size of procyanidins is defined by the degree of polymerization (DP). We have reported that the average DP of procyanidins in sorghum was 8.4 (*6*) and consisted of catechin (88%) and epicatechin (12%) as the chain-terminating units. The chain extension units were exclusively epicatechin (*3, 6*). Procyanidins in sorghum are concentrated in the bran layer, which can be removed by abrasive milling (decortication) (*7*). Over 60% of procyanidins in sorghum are polymers with DP > 10 (*3, 8*).

Catechins or procyanidins from other food sources are reported to have positive health effects in humans including cancer and cardiovascular disease prevention and antidiabetic effects (reviewed in ref *9*). However, the bioavailability of

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catechins and procyanidins appeared to be low (*10*). Cereal processing, such as extrusion, can affect the total procyanidin content as well as the proportion of oligomers and polymers in the total (*8, 11*). Therefore, we postulated that extrusion may affect the bioavailability of catechins and procyanidins in sorghum.

MATERIALS AND METHODS

Terms. Catechin refers to (+)-catechin or (−)-catechin. Epicatechin refers to (+)-epicatechin or (−)-epicatechin, because these epimers are interchangeable (*12*) and could not be separated and identified with the chromatographic methods used in these studies. Catechins refer to the catechin, epicatechin, or their methylated forms. The sum of these was called total catechins. Procyanidins refer to the oligomers (DP 2–10) and polymers (DP > 10) of catechin and epicatechin.

Chemicals. (±)-Catechin, (−)-epicatechin, and α-amylase (type VI-B from porcine pancreas, 29 units/mg) were purchased from Sigma Chemical Co. (St. Louis, MO). 3′-*O*-Methylcatechin, 4′-*O*-methylcatechin, 3′-*O*-methylepicatechin, and 4′-*O*-methylepicatechin were synthesized by methylating (±)-catechin or (−)-epicatechin with methyl iodine according to a published method (*13*). They were purified by preparative HPLC, and the methylation position was confirmed with tandem mass spectrometry (*14*). Procyanidin dimer B1 was purchased from ChromaDex (St. Santa Ana, CA). Procyanidin composite oligomer standard (DP = 1–10) and a polymer standard (DP = 36.1) were prepared as described previously (*3*).

Sorghum Extrusion. Sorghum was extruded using a single-screw extruder (model MX-300i, Maddox Metal Works Inc., Dallas, TX). The moisture content of whole sumac sorghum grain was adjusted to 14%, mixed with sumac sorghum bran (1:1), and allowed to sit overnight before extrusion. The conditions of extrusion included the following: spindle rpm, 295; heater temperature, 164 °C; die speed, 40 rpm; amps, 23.4. The extruded product was ground through a Wiley mill before being given to the pigs.

Animals and Diet. Healthy pigs (Hampshire:Duroc cross, $n = 6$) were purchased from a local swine producer and brought to the Arkansas Children's Nutrition Center animal facility and allowed to adapt for a period of 7 days before surgery. All animal procedures were approved by the Animal Care and Use Committee (ACUC) of the University of Arkansas for Medical Sciences. On day 8, surgery was performed using isoflurane as anesthetic during which a catheter (silastic tubing, 100 cm long; i.d., 1.02 mm; o.d., 2.16 mm, Dow Corning) was implanted into the femoral artery. The catheter was filled with heparinized saline (1000 units/L) and was flushed with saline every other day and filled with heparinized saline. After surgery, the pigs were allowed 7 days to recover. Four days before administration of the sorghum, the pigs were fed a purified diet that was free of any polyphenolic or flavonoid-like compounds (*15*). At the time of blood sampling, the pigs weighed 8.9 ± 1.1 kg (mean ± SE). The pigs were placed in a metabolic cage and food-deprived overnight with water freely available before the experiment. A baseline urine sample was collected in the morning. Sorghum powder was mixed with water (1:3, w/w) and was given via gastric intubation. Extruded sorghum formed a sticky slurry after mixing with water, which could not be delivered via gastric tube. This was overcome by adding α-amylase (5% of sorghum w/w) in the mixture. Pigs were randomized, and each pig received sumac sorghum, extruded sumac sorghum, or white sorghum on three occasions in the same dose (7 g of sorghum/kg$^{0.75}$). All sorghums were a mixture of 50% whole grain and 50% bran. Immediately before feeding, a 0 h blood sample was taken from the catheter. Urine samples were collected from pigs before and between 0 and 2, 2 and 4, and 4 and 24 h after consumption of the sorghum. Blood was drawn from the catheter at 1, 2, and 4 h after feeding into tubes containing heparin as anticoagulant. Tubes were centrifuged at 3000 rpm for 10 min to collect plasma. All samples were stored at −20 °C prior to analyses.

Analytical Methods. *Catechin, Epicatechin, and Procyanidins in Sorghum.* Catechin and epicatechin coelute on normal phase HPLC and thus could not be quantified. They were analyzed using reverse phase HPLC with fluorescence detection. Separation was performed on a Phenomenex Synergi Max-RP column (250 × 4.6, 4 μm, Phenomenex, Torrance, CA) using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA). The solvent consisted of (A) 0.1% (v/v) of formic acid in water and (B) methanol. The 23.5 min linear gradient was as follows: 0–17.5–18–23–23.5 min, 22–35–80–80–22% of B, followed by 7 min of re-equilibration of the column before the next run. The flow rate was 0.8 mL/min. The fluorescence detector was set at 276 nm for excitation and at 316 nm for emission. Catechin and epicatechin eluted at 9.7 and 14.5 min, respectively. Procyanidins were quantified using normal phase HPLC with fluorescence detection as described previously (*3, 8*).

Catechins and Procyanidin Dimers in Urine and Plasma. Thawed urine (100 μL) was added into 6 mL screw-capped glass tubes. Four hundred microliters of ammonium acetate buffer (pH 5, 0.5 M) containing 80 units of sulfatase (type H1, from *Helix pomatia*, Sigma, St. Louis, MO) and 1 mM of ascorbic acid was added. Tubes were incubated in a 37 °C water bath for 45 min. Twenty-five microliters of 6 N HCl was added into the tube to adjust the pH to 2–3. Catechins and procyanidin dimers were extracted twice with 2.5 mL of ethyl acetate. To the combined ethyl acetate extracts was added 50 μL of 100 mM ascorbic acid solution. Solvent was evaporated on a SpeedVac (SC210A; Thermo, Marietta, OH) at 25 °C. Dried extracts were reconstituted in 500 μL of 30% methanol containing 2 mM ascorbic acid. For plasma, 400 μL was used for extraction. Acetic acid (100 μL) was used in the place of HCl to adjust the pH. After the incubation, tubes were extracted twice with 2.5 mL of hexane to remove the lipids before ethyl acetate extraction.

Catechins in urine or plasma were analyzed on an Agilent 1100 HPLC system coupled to an API 4000QTRAP linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA). Separation was carried out on a Phenomenex Synergi Max-RP column (150 × 3.0, 4 μm) using a flow rate of 0.4 mL/min. The solvent consisted of (A) 0.1% (v/v) of formic acid in water and (B) methanol. The 17.5 min linear gradient was as follows: 0–10–12–17–17.5 min, 32–60–80–80–90–32% of B, followed by 7 min of re-equilibration of the column before the next run. The mass spectrometer used an electrospray interface in positive ionization mode. Other parameters for the mass spectrometer included the following: declustering potential, 60 V; collision energy, 23 V; curtain gas, 20 psi; temperature, 450 °C; ion spray voltage, 4500 V; dwell time, 300 ms. Catechins were quantified using their specific fragments: 291.2 > 139.1 *m/z* for catechin and epicatechin; and 305.2 > 139.1 *m/z* for 3′-*O*-methylcatechin, 4′-*O*-methylcatechin, 3′-*O*-methylepicatechin, and 4′-*O*-methylepicatechin. Catechins were quantified against external standards. The linear standard curves had an accuracy of 92–103% in a concentration range of 5–800 ng/mL. This method had a quantitation limit of 8 pg injected on column and intra-assay variation of 5%.

Detection of procyanidin dimers in plasma and urine was performed on the same LC-MS system. The 15.5 min linear gradient was as follows: 0–7–10–15–15.5 min, 25–55–80–90–25% of B, followed by 7 min of re-equilibration of the column before the next run. Procyanidin dimer showed higher sensitivity in negative ionization mode than positive mode using the electrospray interface; thus, negative mode was used. Other parameters for the mass spectrometer included the following: declustering potential, −130 V; collision energy, −36 V; curtain gas, 20 psi; temperature, 450 °C; ion spray voltage, −4500 V; dwell time, 300 ms. Procyanidin dimers were detected using their specific fragments, 577.2 > 288.8 *m/z*. Procyanidin dimer B1 eluted at 6 min and had a detection limit of 10 pg injected on column.

Statistics. Data are expressed as mean ± standard error. Statistical analyses were performed on Excel (Microsoft Office 2003). Pigs from two diet groups were compared by paired *t* test. A difference of $P \leq 0.05$ was considered to be significant.

RESULTS

The mixture of sumac sorghum bran (50%) and whole grain (50%) contained 36.2 mg of total procyanidins with 26.4 mg/g as polymers (72.7% of the total). Extrusion decreased the total and polymeric procyanidins to 21.5 and 13.9 mg/g, respectively

Table 1. Procyanidin Contents (Milligrams per Gram) in White Sorghum, Sumac Sorghum, and Sumac Sorghum Extrudate[a]

procyanidin	white sorghum	sumac sorghum	sumac sorghum extrudate
catechin	ND[b]	0.07 ± 0.002	0.10 ± 0.00
epicatechin	ND	0.01 ± 0.002	0.02 ± 0.00
dimers	ND	0.59 ± 0.01	0.97 ± 0.01
trimer	ND	0.69 ± 0.01	0.78 ± 0.01
4–6-mers	ND	3.66 ± 0.20	2.96 ± 0.03
7–10-mers	ND	4.88 ± 0.33	2.97 ± 0.04
polymers	ND	26.38 ± 1.94	13.86 ± 0.05
total procyanidins	ND	36.20 ± 2.48	21.54 ± 0.04

[a] All were mixtures of 50% of whole grain and 50% of bran. Data are mean ± SD of duplicate analyses. [b] Not detected.

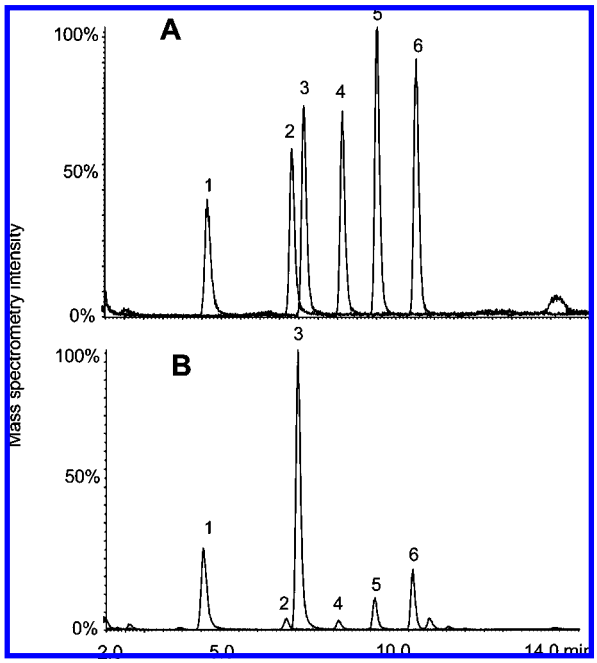

Figure 1. LC-MS/MS profile of catechin standard with a 5 ng/mL at 5 μL injection (**A**) and pig plasma 1 h after feeding sumac sorghum extrudate (**B**). Peaks 1, 2, 3, 4, 5, and 6 are catechin, epicatechin, 3′-*O*-methylcatechin, 4′-*O*-methylcatechin, 3′-*O*-methylepicatechin, and 4′-*O*-methylepicatechin, respectively.

(**Table 1**). The content of 4–6-mers and 7–10-mers also decreased with extrusion. The decrease of total procyanidins by extrusion was likely due to degradation or oxidative polymerization. On the other hand, the contents of monomers (sum of catechin and epicatechin), dimers, and trimers were 0.08, 0.59, and 0.69 mg/g. They increased to 0.12, 0.97, and 0.78 mg/g in the extruded sorghum. Catechins and procyanidins were not detected in white sorghum. Extrusion decreased total and polymeric procyanidins in sorghum and increased the content of lower oligomers.

Catechin, epicatechin, and their methylated metabolites were detected in plasma (**Figure 1**). Catechin and methylated catechin appeared to be the dominant metabolites. Catechin and methylated catechin accounted for 97 and 81% of total plasma catechins in pigs fed sumac sorghum and their extrudate, respectively. Epicatechin and methylated epicatechin appeared to be minor metabolites (**Figure 2**). The content of 3′-*O*-methylcatechin was 10-fold greater than that of 4′-*O*-methylcatechin in plasma, whereas the content of 3′-*O*-methylepicatechin appeared to be similar to that of 4′-*O*-methylepicatechin. Plasma concentrations of catechin, 3′-*O*-methylcatechin, and

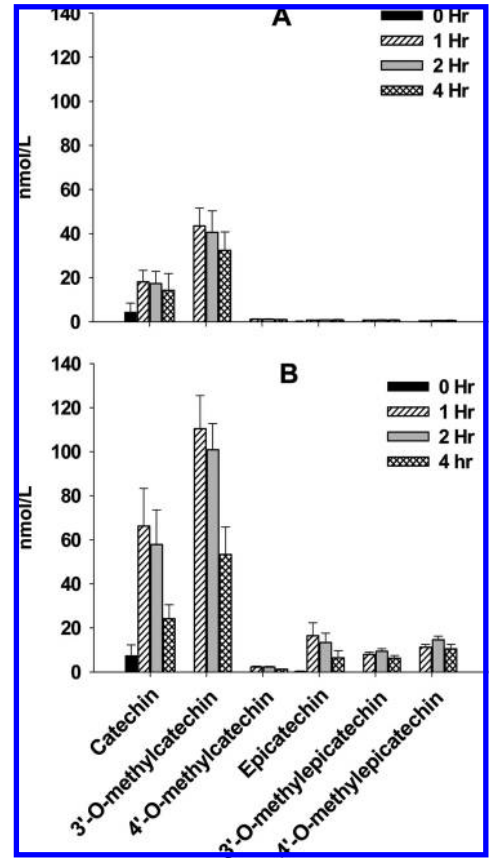

Figure 2. Plasma concentrations of catechins in pigs fed sumac sorghum (**A**) or sumac sorghum extrudate (**B**). Data are mean ± SE, $n = 6$.

total catechins were close to zero at baseline. They increased and peaked at 1 h after sumac sorghum feeding. Their concentrations were significantly higher in pigs fed extruded sumac sorghum than those fed nonextruded sumac sorghum. Plasma concentrations of catechin were close to zero at all time points after feeding of the white sorghum (**Figure 3**).

Catechin and methylated catechin accounted for 95 and 80% of total catechins in the urine of pigs fed sumac sorghum and their extrudate, respectively. The excretion of 3′-*O*-methylcatechin was significantly higher than that of 4′-*O*-methylcatechin in urine, whereas the excretion of 3′-*O*-methylepicatechin appeared to be similar to that of 4′-*O*-methylepicatechin (**Figure 4**). About 70% of catechins in the 24 h urine were excreted in the first 4 h after sumac sorghum feeding. Cumulative excretions of catechin, 3′-*O*-methylcatechin, and total catechins in pigs fed extruded sumac sorghum were significantly higher than those in pigs fed nonextruded sumac sorghum. Catechins were not detected in pig urine after feeding the white sorghum (**Figure 5**).

Procyanidin dimers were not detected in the plasma or urine of pigs after feeding of nonextruded or extruded sorghum.

DISCUSSION

Results from this study indicate that the bioavailability of sorghum catechins was improved by extrusion. Although the absolute difference between catechins (catechin + epicatechin) is small (0.04 mg/g) between nonextruded and extruded sorghum, the percentage increase was 50%. On the basis of the amount of sorghum given to the pigs, this increase would provide 4.7 μmol of catechins, which if 7% or more of this was absorbed, would account for the increase observed in urinary excretion. The increase of catechin concentrations in urine or plasma could be explained by a difference of acces-

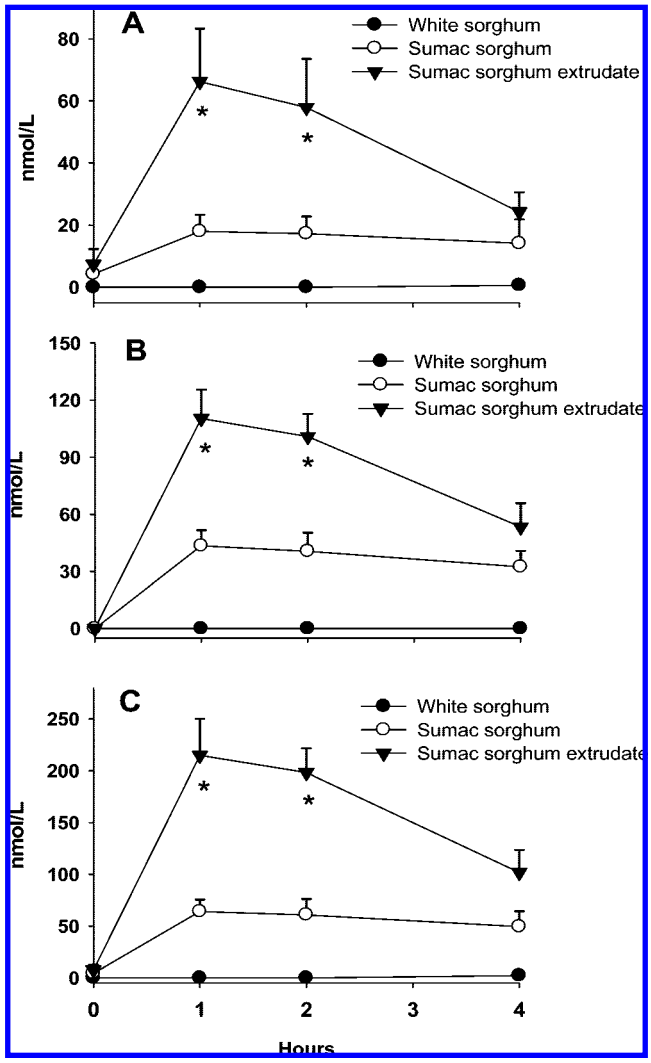

Figure 3. Plasma concentrations of catechin (**A**), 3′-*O*-methylcatechin (**B**), and total catechins (**C**) in pigs fed white sorghum, sumac sorghum, or sumac sorghum extrudate. Data are mean ± SE, $n = 6$. ∗, $P \leq 0.05$ by paired *t* test between sumac sorghum and sumac sorghum extrudate.

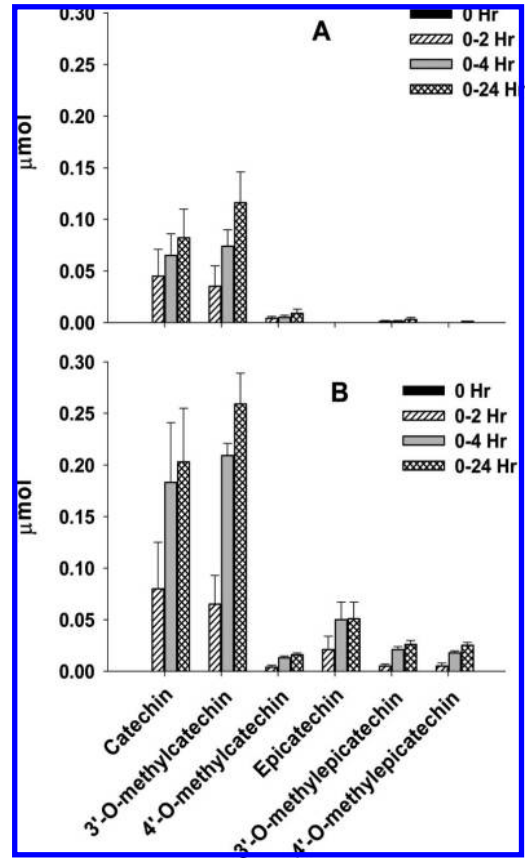

Figure 4. Cumulative urinary excretion of catechins in pigs fed sumac sorghum (**A**) or sumac sorghum extrudate (**B**). Data are mean ± SE, $n = 4$.

sibility of the catechins. We cannot rule out the fact that the amylase treatment may have increased the accessibility of the catechins.

In this experiment the concentration of monomers and lower oligomers increased modestly in the extruded sorghum, but much larger decreases in the concentrations of higher oligomers (DP > 4) were observed. In more recent experiments (Prior et al., unpublished data), we have observed a consistent decrease in concentrations of higher oligomers (DP > 4) and an increase in monomers through trimers. Although lacking direct evidence, this may suggest that heat, high pressure, and shearing force created during extrusion produced depolymerization of procyanidins.

It has been reported that procyanidin monomers, dimers, and trimers can be absorbed, but higher oligomers and polymers are not absorbed (*10*). However, in this study we detected only monomers in plasma and urine. Indeed, procyanidin dimers and trimers have been detected in blood after human or rats consumed procyanidin-rich diets (*16–18*). We did not detect dimers in pig plasma or urine, in part because sorghums contained very low levels of procyanidin dimers and absorption of dimers was lower than the monomers (*16*). However, we cannot preclude that dimers were not absorbed, because part of the absorbed dimers may degrade into constituent catechins in vivo (*19*). In situ perfusion studies demonstrated that procyanidin dimers B2 and B5 were cleaved into constituent monomeric units while transferring across the small intestine and that the resultant epicatechin was the primary bioavailable molecule (*20*).

The procyanidins in sorghum have a unique property in that catechin is present only in the chain termination units. About 88% of the chain termination units were catechin, and the chain extension units were exclusively epicatechin (*3, 6*). Therefore, significant absorption of dimers or trimers would likely increase the proportion of epicatechin in plasma. About 12.5 and 16.7% of the monomers in sumac sorghum and sumac sorghum extrudate were epicatechin. Epicatechin and methylated epicatechin accounted for 3.3 and 18.6% of total plasma catechins (based on area under curve) in pigs fed sumac sorghum and sorghum extrudate, respectively. Thus, absorption of dimers or trimers and degradation into constituent catechins in vivo was suggested but appeared to be minimal.

If it is assumed that all of the absorbed catechins were from the catechins, about 2% of ingested catechin and 0.2% of ingested epicatechin were excreted in 24 h urine in their original and methylated forms in pigs fed sumac sorghum. About 4% of catechin and 4.2% of epicatechin were excreted in 24 h urine in pigs fed extruded sorghum. The fractional excretion of ingested catechin and epicatechin in the 24 h urine was consistent with the ratios observed in rats after long-term sorghum bran feeding (*21*) and in rats (*22*) or humans (*23*) after a single dose of catechin. The concentration of catechin in pig plasma (66 nM) peaked at 1 h after ingestion of extruded sorghum, which was comparable to levels observed in men after

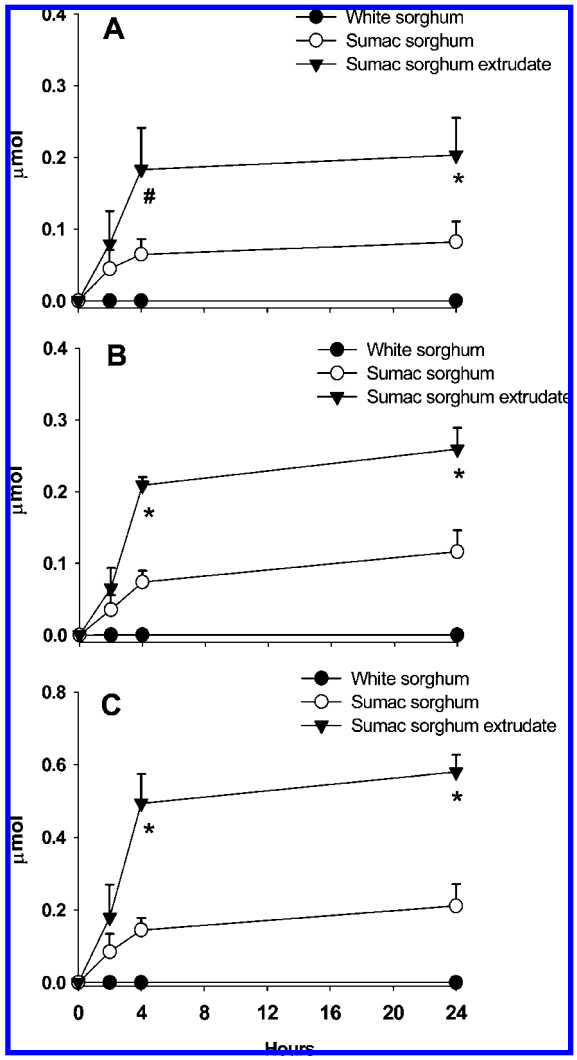

Figure 5. Cumulative urinary excretion of catechin (**A**), 3′-*O*-methylcatechin (**B**), and total catechins (**C**) in pigs fed white sorghum, sumac sorghum, or sumac sorghum extrudate. Data are mean ± SE, $n = 4$. #, $P = 0.1$, and ∗, $P \leq 0.05$, by paired t test between sumac sorghum and sumac sorghum extrudate.

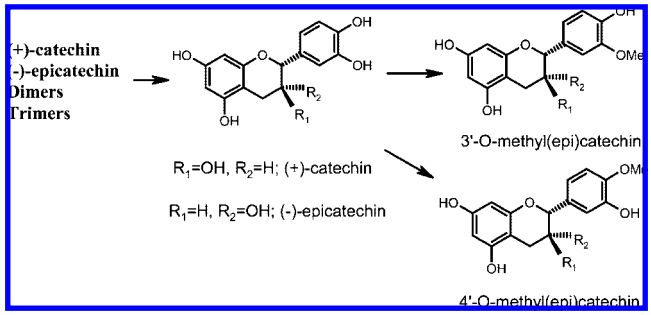

Figure 6. Absorption and metabolism of sorghum catechins and procyanidins.

a single serving of red wine, who had a C_{max} of 77 nM and a t_{max} of 1.3 h (*24*).

The results of our data suggest, but cannot be taken as definite proof, that procyanidins were depolymerized by extrusion. The change in profile of procyanidins and catechin may account for the increase in blood level of catechins. Procyanidins (especially the polymers) bind tightly onto other macromolecules, such as fiber and protein (*25*). The effects of extrusion on this binding are not clear, but may have degraded these macromolecules into smaller fragments, releasing bound catechins or procyanidins.The metabolism of catechins in sorghum is summarized in **Figure 6**. Extrusion increased the content of catechin, epicatechin, dimers, and trimers in sorghum. Catechins can be directly absorbed. If dimers and possibly trimers can also be absorbed, they can be cleaved into catechins while transferring across the intestine (*20*). Catechins can also be methylated by catechol-*O*-methyltransferase in both the small intestine and the liver (*26*). In conclusion, extrusion improved the bioavailability of catechins in sorghum; however, the mechanisms responsible for this improved absorption remain to be determined.

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