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Phytochemicals in Sweet Sorghum (*Dura*) and Their Antioxidant Capabilities against Lipid Oxidation

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ABSTRACT: Hydrophilic (HPE) and lipophilic (LPE) extracts were obtained from the Louisiana sweet sorghum millets. Nine major hydrophilic phytochemicals were quantified at levels of 8.9 μg/g for cinnamic acid to 1570.0 μg/g for apigeninidin. Lipophilic phytochemicals (α- and γ-tocopherol, lutein, and β-carotene) were quantified at levels of 7.7, 145.7, 4.8, and 18.8 μg/g, respectively. The total phenolic contents of HPE and LPE were 768.9 and 97.6 μg of catechin equivalent/g, respectively, while DPPH activities were 6.5 and 0.8 μmol of Trolox equivalent/g for HPE and LPE, respectively. In an emulsion model, HPE exhibited higher capability of inhibiting cholesterol oxidation and stabilizing linoleic acid than LPE. Inhibition rates of cholesterol oxidation for HPE and LPE at 40 μg/mL were 92.2% and 65.4%, respectively. Retention rates of linoleic acid were 70.4% for HPE and 33.6% for LPE at a given concentration. Thus, HPE of sweet sorghum millet has potential in functional food applications.

KEYWORDS: *sorghum, antioxidant, cholesterol, fatty acid, biomass*

INTRODUCTION

Sweet sorghum is widely cultivated around the world and utilized for food or animal feed.[1] In the U.S., sorghum is mainly used for animal feed after the stalks are removed during postharvest processing. Recently, sorghum millets have been found as an economical biofuel material for producing ethanol.[2] This potential application in bioenergy has raised the value of sorghum and led to its increasing production in the U.S. annually. Although the color and texture of sorghum millets are not as desirable as other cereals such as wheat and oats for food applications, it has been reported to possess a variety of antioxidant phytochemicals including polyphenols, anthocyanins, carotenoids, and tocopherols.[3] Daily consumption of these antioxidants could help reduce the risk of developing chronic diseases associated with the oxidation of cholesterol and fatty acids.[4] For hydrophilic antioxidants, they are usually extracted by water or alcohols and leave the major sorghum biomass intact for biofuel production. Therefore, the hydrophilic antioxidants extraction could be a pretreatment in the biofuel production of sorghum. As the extract has great potential in health promoting application, the treatment can increase the economic value of sorghum used as a biofuel material.

The profiles and levels of phytochemicals in sorghum millets are dependent on the varieties and growth environments of sorghums. In this study, antioxidant phytochemicals and their antioxidant activity in the sweet sorghums harvested in Louisiana were identified and evaluated. The results would be helpful in utilizing Louisiana sorghum millets as a potential health promoting source and biofuel material in the meantime. To identify the major phytochemicals responsible for the activity and health benefits of sorghum millets, the hydrophilic and lipophilic phytochemicals in millets were extracted and evaluated individually. In addition to using common antioxidant activity assay methods, the antioxidant capabilities of both extracts were evaluated by using an emulsion model, which consisted of cholesterol and linoleic acid at the same level as found in the human blood serum.

Cholesterol and fatty acids are the primary components in the cell membrane and blood serum of mammals and can be oxidized under oxidative stress. The oxidation of cholesterol and fatty acids results in generation of lipid oxidation products, which are toxic to the endothelial cells of blood vessels and cell membranes. For example, 7-ketocholesterol, the primary cholesterol oxidation product, could display pro-apoptotic, pro-inflammatory activities and cause degenerative diseases or lipid metabolism disorders.[5−7] The fatty acids, especially unsaturated fatty acids, likely undergo lipid peroxidation and yield short chain inflammatory mediators, such as aldehydes and hydroperoxides, which are associated with cellular damage, arthritis, cerebral arteries, and other coronary diseases.[8] Thus, inhibition of cholesterol or fatty acid oxidation in the serum is crucial to maintain normal lipid metabolism and integrity or permeability of cell membranes in the body. However, evidence of the function of antioxidant phytochemicals present in sweet sorghum millets in inhibiting cholesterol and fatty acid oxidation has not been documented. The performance of antioxidants in sorghum millets in the cholesterol and linoleic acid emulsion model could closely reflect their antioxidant activity in the human blood serum.

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MATERIALS AND METHODS

Chemicals and Materials. HPLC grade methanol, hexane, acetic acid, and sodium phosphate dibasic (anhydrous) were purchased from Fisher Chemicals (Fair Lawn, NJ). Sodium phosphate monobasic (anhydrous) was obtained from Amresco (Solon, OH). Isopropanol and sodium bicarbonate were obtained from Mallinckrodt Co. (Paris, KY). Ethyl acetate and hydrochloride acid were purchased from EM Science (Gibbstown, NJ). Tween 20, 2,2-diphenyl-l-picrylhydrazyl (DPPH), Trolox, Folin-Ciocalteau reagent, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), cholesterol, 7-ketocholesterol, linoleic acid, heptadecanoid acid (C17:0), boron trichloride in methabol (BCl_3-methanol), α-tocopherol, γ-tocopherol, apigeninidin-chloride, and all of the phenolic standards (ferulic acid, *p*-coumaric acid, cinnamic acid, catechin, gallic acid, syringic acid, kaempferol, and quercetin) were provided by Sigma-Aldrich (St. Louis, MO). Sweet sorghum millets (*Dura*) were obtained from the Sugar Research Station, Louisiana State University Agricultural Center (St. Gabriel, LA).

Extraction and Determination of the Antioxidants in Sweet Sorghum Millets. The extraction method was described in the study of Jang and Xu.[9] The sweet sorghum millets were ground using a kitchen blender. The ground powder was screened by using a 1 mm brass sieve. Ten grams of the sifted powder sample was extracted using 50 mL of acidified methanol (pH 1) twice at 60 °C. The methanol layers were combined and evaporated by a vacuum centrifuge evaporator (Labconco, Kansas City, MO) to obtain the hydrophilic extract (HPE). The same procedure was applied to extract lipophilic compounds in the millet powder by using hexane solvent instead of methanol. Both hydrophilic and lipophilic extracts were weighed (dry weight basis) and used to prepare the stock solution (10 mg/mL) in methanol or hexane, respectively.

Anthocyanins and other phenolic compounds in the hydrophilic extract (HPE) were determined by the HPLC method described by Jang and Xu.[9] Carotenoids in the HPE were determined by the method as described by Sun et al.[10] Tocopherols in the lipophilic extract (LPE) were measured by using a normal phase HPLC system with a fluorescence detector. The HPLC analysis conditions were as described by Jang and Xu.[9]

Determination of Total Phenolic Contents of the Hydrophilic and Lipophilic Extracts. The total phenolic content method was described in the study of Jang and Xu.[9] The 10-fold diluted Folin-Ciocalteau reagent (0.75 mL) was reacted with 0.1 mL of the extract stock solution. After 5 min, 0.75 mL of sodium bicarbonate (60 g/L) was added to the mixture and incubated at 25 °C for 90 min. The absorbance was recorded by a UV−visible SpectraMax Plus384 spectrophotometer (Molecular Devices, Sunnyvale, CA) at 750 nm. Catechin (0.05, 0.1, 0.2 mg/mL) was used to plot a standard curve. The total phenolic content of the extract was expressed as milligram of catechin equivalent/gram of sweet sorghum millets.

Determination of Antioxidant Activities of the Hydrophilic and Lipophilic Extracts Using DPPH Method. The DPPH method was carried out using a modification of the procedure by Yue and Xu.[11] DPPH solution (0.1 mM, 1.8 mL) was mixed with 0.2 mL of the hydrophilic extract stock solution or the lipophilic extract stock solution, which was first evaporated to dryness and then redissolved with 0.2 mL of methanol. The reaction was carried out at 25 °C for 30 min in the dark. The absorbance of the mixture at 515 nm was monitored at zero time (Ab_0) and after 30 min incubation (Ab_{30}), respectively. The inhibition rate was calculated as:

$$\text{inhibition rate } (\%) = [(Ab_0 - Ab_{30})/Ab_0] \times 100$$

Different concentrations of Trolox (0.05, 0.10, 0.20, and 0.50 mM) versus their corresponding inhibition rates were plotted to obtain a standard curve. The inhibition rate of the testing sample was calculated and converted to μmol Trolox equivalent/gram of sweet sorghum millets.

Preparation of Cholesterol−Linoleic Acid Oxidation Emulsion. The cholesterol−linoleic acid oxidation emulsion consisted of cholesterol (1000 μg/mL), linoleic acid (500 μg/mL), AAPH (300 μg/mL), and Tween 20 (10 μL/mL) in phosphate buffer solution (PBS, pH 7.2). The emulsion was homogenized by a microfluidizer materials processor (M-110P, Microfluidics, Newton, MA). The hydrophilic or lipophilic extract stock solution (40 or 80 μL) was added to a 40 mL vial and dried. It then was mixed and homogenized with 20 mL of the emulsion, resulting in the antioxidant-containing emulsion having either 20 or 40 μg/mL of the extract. The emulsion without the extract served as a blank group. Each treatment and the blank group had three replications. All of the vials were incubated in a 37 °C water bath and continually agitated by a multiple magnetic stirrer (Multistirrer, VELP Company, Italy) for 48 h.

Extraction and Determination of 7-Ketocholesterol. One milliliter of the emulsion sample was collected from each vial at different time (0, 24, and 48 h) and mixed with 2 mL of hexane. The solution was vortexed and centrifuged at 5000*g* for 15 min. The upper hexane layer was separated and transferred to a clean test tube with 0.1 g of anhydrous $NaSO_4$. The primary oxidation cholesterol product, 7-ketocholesterol, was analyzed by an HPLC with normal phase column (i.d. 250 × 4.60 mm 5 μm, Supelco, Bellefonte, PA) and a UV detector. The mobile phase composition and gradient program were described in the studies by Xu et al. and Zhang et al.[12,13] The inhibition rate of the extract for preventing cholesterol oxidation at each sampling time was calculated using the following equation:

$$\text{inhibition rate } (\%) = [(C_b - C_t)/C_b] \times 100$$

where C_b was the concentration of 7-ketocholesterol in the blank at a sampling time, and C_t was the concentration of 7-ketocholesterol in the treatment group at the same sampling time.

Extraction and Determination of Linoleic Acid. One milliliter of the emulsion sample was collected and mixed with 2 mL of hexane containing internal standard C17:0 (100 μg/mL). The hexane supernatant was separated by centrifugation and transferred to a clean test tube. The hexane was evaporated by nitrogen flow to obtain dried sample. Two milliliters of BCl_3−methanol was added to the dried sample for esterification at 60 °C for 30 min. Next, 1 mL of water and 1 mL of hexane were mixed with the reaction solution and vortexed. After centrifugation, the upper hexane layer was mixed with anhydrous $NaSO_4$ and transferred to a GC vial. The operating condition of GC analysis was as described by Zhang et al.[13] The retention rate of linoleic acid in the emulsion was calculated as follows:

$$\text{retention rate } (\%) = (C_t/C_0) \times 100$$

where C_0 was the concentration of linoleic acid at 0 h, and C_t was the concentration of linoleic acid at 24 or 48 h in the same emulsion.

Data Analysis. The concentrations of bioactive components, total phenolic contents, and antioxidant activities of each extract were expressed as the mean and standard error from three independent extractions and analysis. The inhibition rate of cholesterol and retention rate of linoleic acid were expressed as the mean and standard error of three replications as well. All calculations were done using Microsoft Excel (Redmond, WA), and one-way ANOVA was used to evaluate significant differences between treatment means using the statistical analysis software (SAS, 9.1.3, Cary, NY).

RESULTS AND DISCUSSION

Antioxidant Phytochemicals in Sweet Sorghum Millets. In addition to its protein, starch, and other macronutrients related to the body energy supply, sorghum millet offers a rich source of antioxidant phenolics with potential health benefits of preventing various chronic diseases. However, the profile and levels of phenolics in a crop usually vary by crop variety and growth environment.[1] In this study, sweet sorghum (*Dura*) samples were harvested in Louisiana where annual average climate temperatures are much higher than in most traditional sorghum growing areas. The local environmental factors such as the higher average temperature (25 °C) or the average rainfall (5−6 in./month) during growing season (May to October) may result in the antioxidant

profiles and levels of Dura sorghum millets that differ from those harvested in cold and dry areas. In our study, the total phenolic content of HPE was 768.9 ± 46.7 μg of catechin equivalent/g of sorghum millets, which was 8 times higher than that of LPE (97.6 ± 8.2 μg of catechin equivalent/g of sorghum millets) (Table 1).

Table 1. Total Phenolic Content and DPPH Free Radical Scavenging Capability (TEAC) of Hydrophilic (HPE) and Lipophilic (LPE) Extracts from the Sweet Sorghum Millets

	HPE	LPE
total phenolic content (μg of catechin equiv/g)	768.9 ± 46.7	97.6 ± 8.2
TEAC (μmol of trolox equiv/g)	6.5 ± 0.1	0.8 ± 0.1

The dominant phenolic sorghum millets was apigeninidin at a level of 1.57 mg/g, which is lower than that reported in black (4.0−9.8 mg/g) and red (3.3 mg/g) sorghum bran by Awika and Rooney.[14] The grain bran usually contains a higher level of antioxidant phenolics than its kernels. Other phenolics such as ferulic, *p*-coumaric, cinnamic, gallic and syringic acid, catechin, kaempferol, and quercetin were identified and quantified in the sorghum millet sample (Table 2). The level of catechin was

Table 2. Concentrations of Bioactive Components in Sweet Sorghum Millets

name	concentration (μg/g)
ferulic acid	107.6 ± 10.1
p-couramic acid	17.9 ± 1.4
cinnamic acid	8.9 ± 0.5
catechin	144.9 ± 3.7
gallic acid	130.6 ± 8.1
syringic acid	38.8 ± 4.0
kaempferol	133.7 ± 6.7
quercetin	22.1 ± 0.9
apigeninidin	1570.0 ± 9.3
lutein	4.8 ± 0.2
β-carotene	18.8 ± 1.1
α-tocopherol	7.7 ± 0.7
γ-tocopherol	145.7 ± 12.7

144.9 ± 3.7 μg/g and much higher than that in the millets reported (6.2−84.7 μg/g) by Bröhan et al.[15] Also, kaempferol, gallic, and ferulic acid were the major phenolics in the sweet sorghum millets at levels of 133.7 ± 6.7, 130.6 ± 8.1, and 107.6 ± 10.1 μg/g, respectively. In a previous study, the levels of gallic and ferulic acid ranged from 13.2−46.0 and 8.9−95.7 μg/g, respectively, in different sorghum varieties.[3] The differences in phenolic composition and concentration of the sweet sorghum from other studies may be due to differences in the light intensity, relative humidity, an average temperature of growing environment, as well as the genetic background of the sorghum species. The combination of these phenolics has been reported to have pharmacological potential in preventing cell inflammatory and cardiovascular diseases.[16,17] Although the carotenoids in fruits and vegetables and their health benefits in preventing retina-aging and prostate and breast cancer are widely reported, their levels and profile in sweet sorghum millets have not been well documented. Concentration of lutein and β-carotene in the sorghum millets was of 4.8 ± 0.2 and 18.8 ± 1.1 μg/g, respectively (Table 2). Vitamin E, α-, β-, γ-, and δ-tocopherol have been reported in red and white sorghum millets. However, only α- and γ-tocopherol were detected in our sorghum samples. The level of γ-tocopherol was 145.7 ± 12.7 μg/g and approximately 100 times higher than in red and white sorghum.[18] Vitamin E, especially γ-tocopherol, could be the major contributor to the antioxidant activity of LPE.

Evaluation of Antioxidant Activities of the Hydrophilic and Lipophilic Extracts. In the DPPH assay, the antioxidant activity of HPE was 6.5 ± 0.1 μmol of Trolox equivalent/g of sorghum millets, while it was only 0.8 ± 0.1 μmol of Trolox equivalent/gram of sorghum millets for LPE (Table 1). Although the DPPH assay is a common method for evaluating the antioxidant activity of phenolics in plant extracts, the absorbance at 515 nm for measuring DPPH change is prone to the interference by other components, such as anthocyanins in the extract, which has absorbance in that wavelength range as well. Thus, the DPPH method is not an ideal assay for determining the antioxidant activity of anthocyanins-rich extract. As the antioxidant activity determined by DPPH assay is the activity of quenching free radicals or H-donor capability of the antioxidant, it may not be actually associated with the required antioxidant function in the body. In fact, not only the activity of an antioxidant but also the properties of the surrounding media and lipid substrates could affect the performance of antioxidant capacity.

In our study, cholesterol and fatty acids, which were the key protecting targets of a health promoting antioxidant, were used as the substrates in the antioxidant activity evaluation model. As compared to the DPPH assay, the activity obtained in this model is much correlated to the capability of the antioxidant in stabilizing lipids in a biological system and preventing hyperlipidemia stress. In this study, an in vitro cholesterol−linoleic acid emulsion model, which simulated the blood serum environment, was used to determine antioxidant activity of the sorghum millet extracts. Cholesterol is an important component of LDL and HDL in the serum. Cholesterol and linoleic acid are also the major lipids of the cell membrane. However, they are vulnerable to oxidation under oxidative stress or attack by free radicals. During oxidation, the generation and accumulation of cholesterol oxidation product 7-ketocholesterol could lead to plague formation in the blood vessel wall and development of coronary atherosclerosis.[19] Also, linoleic acid could be oxidized to produce cytotoxic short chain aliphatic compounds.[20] In the cholesterol−linoleic acid emulsion, an increase in 7-ketocholesterol and decrease in linoleic acid were measured to assess the degree of cholesterol and fatty acid oxidation in the system, individually.

After 24 h oxidation, 7-ketocholesterol in the blank increased to 2.85 μg/mL from undetectable levels (Figure 1). Meanwhile, the treatments with 20 and 40 μg/mL of LPE had 7-ketocholesterol at 1.86 and 1.48 μg/mL, respectively. For the two HPE treatments, the level of 7-ketocholesterol was below 1 μg/mL. After 48 h oxidation, HPE showed higher antioxidant activity with an inhibition rate of 88.5% for HPE1 (20 μg/mL) and 92.2% for HPE2 (40 μg/mL), while it was 45.2% and 65.4% for LPE1 (20 μg/mL) and LPE2 (40 μg/mL), respectively (Figure 1). For linoleic acid oxidation, only 33.6% of linoleic acid was retained in the blank after 24 h oxidation. The retention rate from low to high was in the order of LPE1 40.1%, LPE2 45.7%, HPE1 61.7%, and HPE2 83.3% (Figure 2). After 48 h oxidation, the retention rate of linoleic acid in the blank was only 15.5%. HPE2 still exhibited the highest retention rate (70.4%) as compared to HPE1 (52.8%),

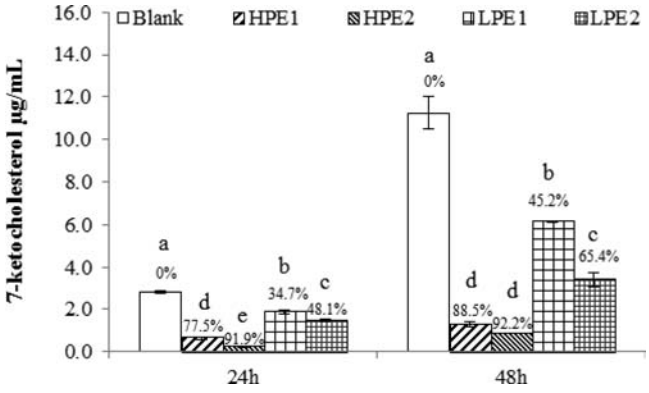

Figure 1. The 7-ketocholesterol levels and cholesterol oxidation inhibition rates in blank, HPE1 (20 μg/mL), HPE2 (40 μg/mL), LPE1 (20 μg/mL), and LPE2 (40 μg/mL) after 24 and 48 h oxidation. *Different letters represent significant differences among the five treatments at $P < 0.05$.

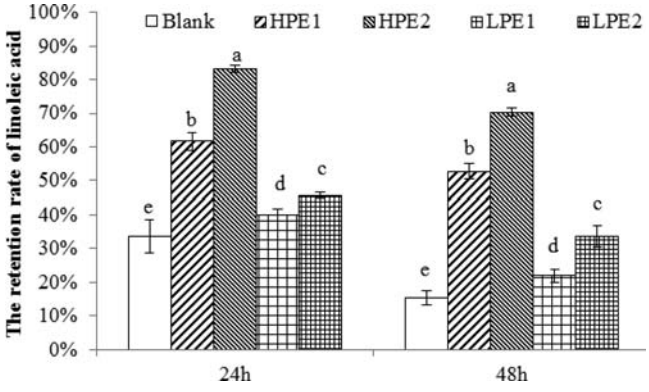

Figure 2. The retention rates of linoleic acid in blank, HPE1 (20 μg/mL), HPE2 (40 μg/mL), LPE1 (20 μg/mL), and LPE2 (40 μg/mL) after 24 and 48 h oxidation. *Different letters represent significant differences among the five treatments at $P < 0.05$.

LPE1 (22.0%), and LPE2 (33.6%). Although both HPE and LPE exhibited inhibiting activity against cholesterol and linoleic acid oxidation in a dose-dependent property, HPE had higher antioxidant activity than LPE in the emulsion model. The abundant apigeninidin and other phenolics could be responsible for the high antioxidant activity of HPE. Most of the hydrophilic phenolics possess at least two hydroxyl groups that could greatly contribute to the protection of lipid substrates from oxidation. The results (Figure 1) are in agreement with the study of Zhang et al.[13] In that study, hydrophilic anthocyanins extract had a higher capability in inhibiting cholesterol oxidation than tocol extract did. Because apigeninidin has multiple cyclic rings and its molecular structure is similar to that of cholesterol, it may have better accessibility than other long carbon chain antioxidants in contacting and protecting cholesterol against oxidation. Although LPE had a high level of γ-tocopherols, the lower level and diversity of other phenolics in LPE were responsible for the lower overall capability in stabilizing lipid. In general, HPE played an important role in preventing lipid oxidation in the emulsion system. In other words, the antioxidant capacity of the sweet sorghum millets is mainly contributed by the hydrophilic phenolic antioxidants.

In this study, the levels and profiles of hydrophilic and lipophilic antioxidant phytochemicals in sweet sorghum millets were evaluated. The dominant hydrophilic and lipophilic antioxidants were apigeninidin and γ-tocopherol, respectively. The hydrophilic antioxidants showed higher antioxidant activity than lipophilic antioxidants in both the DPPH assay and the cholesterol−linoleic acid oxidation model. Therefore, the health benefits of sweet sorghum may be mainly attributed by its hydrophilic antioxidant phytochemicals. Thus, with its high biomass and diverse antioxidants, sweet sorghum (*Dura*) could be a valuable biomaterial for both biofuel and health promoting applications.

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Notes

The authors declare no competing financial interest.

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