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ARTICLE

Policosanol, α-Tocopherol, and Moisture Content as a Function of Timing of Harvest of Switchgrass (*Panicum virgatum* L.)

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Using switchgrass (*Panicum virgatum* L.) as a cellulosic feedstock for the production of ethanol could lead to the extraction of co-products prior to the pretreatment step, thereby adding value to the ethanol conversion process. Policosanols, registered as 142583-61-7, are present in *Poaceae* and are a mixture of long-chained primary alcohols. Policosanols are composed mainly of docosanol (C_{22}), tetracosanol (C_{24}), hexacosanol (C_{26}), octacosanol (C_{28}), triacontanol (C_{30}), and dotriacontanol (C_{32}). This study determined changes in moisture, policosanol, and α-tocopherol concentrations of Cave-in-Rock and Blackwell switchgrass cultivars during maturation from July to December in Arkansas and Oklahoma. Moisture content on a dry weight basis declined from 150 to 50% with progressive harvests. The total policosanol concentration ranged between 89 mg/kg for July harvested Cave-in-Rock switchgrass from Arkansas and 182 mg/kg for August harvested Cave-in-Rock switchgrass for Oklahoma, and these values remained relatively constant throughout the season. This is the first report on the presence of policosanols in switchgrass. Total switchgrass policosanol concentrations were lower than those typically reported for sorghum grains; however, switchgrass-extracted policosanols contained different policosanol ratios, wherein C_{30} and C_{32} alcohol ranges were 36−41 and 43−50%, respectively. α-Tocopherol extracted from both switchgrass cultivars varied between 320 and 400 mg/kg but decreased in the October harvest after frost.

KEYWORDS: *Panicum virgatum*; switchgrass; policosanol; α-tocopherol; moisture content

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass native to North America, which has potential as a major cellulosic feedstock source for ethanol production. Biomass-derived liquid fuel has the potential to displace 30% of U.S. petroleum consumption (*1*). In the saccharification platform, cellulosic biomass is pretreated to liberate the cellulose and hemicellulose from lignin and enzymatically hydrolyzed to sugars, which can then be fermented into ethanol. On-farm studies have indicated that switchgrass can produce output energy that is 6.4-fold greater than the input energy supplied to grow and harvest the biomass, thus giving credence to the concept of cellulosic ethanol (*1*).

Switchgrass also has potential to provide high-value phytochemicals prior to or during the pretreatment step, thereby adding value to the ethanol conversion process. Switchgrass has been reported to contain volatile compounds, such as 6-methyl-5-hepten-2-ol, *cis*-3-hexenol, 1-octen-3-ol, 2-nonanol, linalool, and borneol (*2*). As a member of the *Poaceae* (grass) family, switchgrass likely contains policosanols. Policosanols are long-chained primary alcohols comprising mainly docosanol (C_{22}), tetracosanol (C_{24}), hexacosanol (C_{26}), octacosanol (C_{28}), triacontanol (C_{30}), and dotriacontanol (C_{32}) (*3*). Numerous other materials have been reported to contain policosanols, including grain sorghum (*Sorghum bicolor*) (*4*), perilla seeds (*Perilla frutescens*) (*5*), sugar cane (*Saccharum officinarum* L.) (*3*), wheat (*Triticum aestivum*), beeswax (*3*), and dried distillers grains (*4*). Grain sorghum has been reported to contain a high content of waxy materials and policosanols in free, nonesterified forms (*4*). Sorghum-extracted policosanols are distributed by carbon chain length as follows: 0−1% C_{22}, 0−3% C_{24}, 6−8% C_{26}, 1% C_{27}, 43−47% C_{28}, 1−2% C_{29}, 40−43% C_{30}, and 1−4% C_{32} (*4*).

The C_{28} and C_{30} policosanols are the two most abundant alcohols in plant materials. In wheat straw, 85% of the total policosanol content is C_{28} (*3*), while more than 40% of the total policosanol content of beeswax is C_{30} (*3*). Policosanols have been reported to improve blood lipid levels, reduce

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platelet aggregation, and improve and increase exercise performance and muscle endurance in coronary heart disease patients (*3*). Currently, policosanols are being consumed to reduce low-density lipoprotein (LDL) levels, while increasing high-density lipoprotein (HDL) levels (*6*). Policosanols are currently available as dietary supplements. Reports suggest that 5−20 mg per day of a mixture of C_{28} and C_{30} alcohols can reduce LDL cholesterol levels by 21−29% and increase HDL cholesterol levels by 8−15% (*7*).

The objective of this study was to determine the policosanol content of two switchgrass varieties that were grown in Fayetteville, Arkansas, and Stillwater, Oklahoma, as a function of harvest time. Obtaining information on the available policosanol concentrations will enable primary speculations as to the feasibility of the possible extraction of these phytochemicals when coupled to conversion to biofuels.

MATERIALS AND METHODS

Cultivation and Handling. Two switchgrass cultivars, "Cave-In-Rock" and "Blackwell", were included in the study. These two cultivars are upland ecotypes (*8*). The switchgrass plots were established from seed in 2001 and grown under dryland conditions at the University of Arkansas Agricultural Research and Extension Center (Fayetteville, AR; Captina silt loam soil) and at Oklahoma State University (Stillwater, OK; Kirkland silt loam). Individual plots were sized 1.7 × 1.8 m and 1.5 × 4.6 m, respectively. No fertilizer was applied in 2006, but in previous years, the plots had received 100 kg/ha of nitrogen as ammonium nitrate. A full description of the plots, which was part of a U.S. study on latitude and longitude effects on switchgrass growth, was reported by Casler et al. (*9*). The plot design was originally a Latin square with six populations and six replications; however, for this experiment, it was treated as a randomized complete block design with two populations and six replications per location.

Switchgrass was sampled at 4 week intervals from the 7th of July until the 13th of December. A total of 12 randomly selected stems were clipped to a 15 cm stubble height and bulked into one sample. Samples harvested at Stillwater, OK, were immediately shipped overnight to Fayetteville, AR, for processing. All samples were weighed and frozen at −20 °C before being loaded into a Freezone Labconco freeze-dryer (Kansas City, MO) for 72 h. When dried, the samples were again weighed. The moisture concentration was calculated on a dry weight basis as (fresh weight − dry weight)/dry weight and expressed as a percentage.

Analytical Procedures. *Standards and Chemicals*. Policosanol and α-tocopherol reference compounds were purchased from Sigma (St. Louis, MO) and VWR International (West Chester, PA), respectively. Hexane, which was used in the extraction process, was obtained from VWR International (West Chester, PA). High-performance liquid chromatography (HPLC)-grade chloroform, which was used for dilution, was acquired from EM Science (Gibbstown, NJ). *N*-Methyl *N*-trifluoroacetamide was obtained from Pierce, Inc. (Rockford, IL).

Sample Preparation/Derivatization. Dry switchgrass samples were ground to a particle size of 4 mm, according to ASAE Standard S319 (*10*). A total of 5 g of the ground sample was weighed and placed in a single thickness Whatman thimble (VWR International, West Chester, PA). The sample was refluxed in a Soxhlet apparatus with 200 mL of hexane for 45 min at 70 °C. A total of 50 mL of the extract was then evaporated at reduced pressure using a rotary evaporator (Yamato, BM 200 Yamoto Scientific, Japan) to approximately 1 mL. The 1 mL concentrate was then transferred to a glass vial and brought to dryness using nitrogen under an evaporation unit. To the dry residue, 0.7 mL of chloroform and 0.3 mL of the derivatizing agent *N*-methyl *N*-trifluoroacetamide (Pierce, Inc., Rockford, IL) were added. The samples were then heated at 70 °C for 20 min to complete the derivatization reaction. A total of 100 μL was taken from the derivatized sample and diluted with chloroform to obtain a final volume of 1 mL.

Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. A total of 1 μL of the derivatized sample was injected (splitless mode) into an Agilent 6890N gas chromatograph/mass spectrometer (GC/MS) (Agilent Technologies, Inc., Wilmington, DE). Hydrogen was used as the carrier gas, flowing at a rate of 1.2 mL/min on a DB-5MS (25 m length × 0.2 mm inner diameter × 0.33 μm film thickness) 128-5522 J&W Scientific column (Agilent Technologies, Inc., Wilmington, DE). The injector temperature was 300 °C, and the total run time was 22 min. The oven temperature was initially 45 °C and was ramped at 20 °C/min to 310 °C and held for 8.75 min. Detection was obtained with an Agilent 5973 N quadrapole mass spectrometer in the positive mode, scanning from m/z 15 to 550. The chromatograms were analyzed by comparing component retention times and mass spectra to those obtained using standard compounds. The derivatized octacosanol compound spectra was also compared to those in the NIST 2 mass spectrometry library for confirmation. When the authenticity of the compounds was established, their concentrations were determined with respective calibration curves.

Statistical Analysis. Differences between locations, cultivars, and harvesting months for phytochemicals and moisture content were tested using analysis of variance (ANOVA) with the general linear model procedure of JMP (SAS Institute, Cary, NC). Each cultivar was replicated 6 times within each location, and the harvest time effect was analyzed as a repeated-measure factor. *F* tests for main effects and interactions were calculated from appropriate error terms.

RESULTS AND DISCUSSION

Changes in switchgrass moisture content as a function of sampling date, cultivar, and location are presented in **Figure 1**. Moisture contents of Cave-in-Rock grown in Arkansas were highest in September and decreased throughout the fall. Biomass sampled from both cultivars from the Oklahoma plots displayed the highest moisture contents at the onset of the sampling period in July.

Reference standards of hexacosanol (C_{26}), octacosanol (C_{28}), and triacontanol (C_{30}) eluted at 13.8, 14.9, and 16.2 min, respectively, as shown in **Figure 2A**. A mass spectral library search was conducted to confirm the presence of this dotriacontanol (C_{32}). Parts **B** and **C** of **Figure 2** present chromatograms of Oklahoma- and Arkansas-grown Cave-in-Rock switchgrass extracts, respectively. This is the first report on the presence of policosanols in switchgrass.

The policosanol concentrations of Cave-in-Rock and Blackwell cultivars were determined as a function of harvest date in Arkansas and Oklahoma, as shown in **Table 1**. For both Arkansas-grown varieties, the C_{26}, C_{28}, C_{30} and C_{32} concentrations remained relatively constant over time. The lowest total policosanol content, 89.5 ± 11.4 (average ± standard deviation) mg/kg, was obtained for July-harvested Cave-in-Rock samples from Arkansas, while the highest, 107.4 ± 12.9 mg/kg, was obtained for October-harvested Cave-in-Rock samples. In Arkansas, there was no significant difference ($p > 0.05$) in the concentrations of total policosanol between varieties grown or based on date of harvests ($p > 0.05$). In contrast, Oklahoma-grown Cave-in-Rock had a greater ($p < 0.05$) policosanol concentration than Blackwell and concentrations were lowest in December ($p < 0.0001$). There was no significant month × variety interaction ($p > 0.05$) at either location.

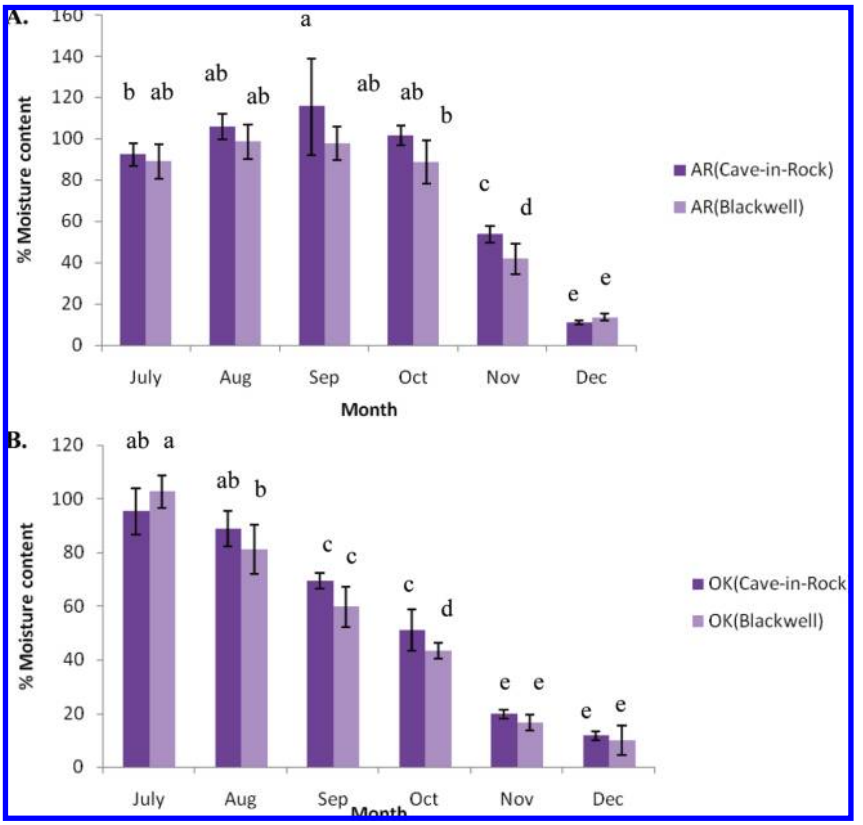

Figure 1. Moisture content of switchgrass as a function of the month of sampling, location, and cultivar. Error bars represent the standard deviations. Bars with identical letters are not significantly different. Bars with non-identical letters are significantly different. (A) Arkansas Cave-in-Rock and Blackwell and (B) Oklahoma Cave-in-Rock and Blackwell.

In comparison to the policosanol content of Arkansas-grown biomass, switchgrass cultivated in Oklahoma displayed a higher total policosanol concentration, ranging from 104.7 ± 24.9 to 182.0 ± 14.5 mg/kg. The total policosanol content in both Oklahoma-grown varieties remained constant until November, but a significant difference ($p < 0.0001$) was observed in December.

For both varieties in both locations, the total policosanol content of switchgrass ranged from 89.5 to 182.0 mg/kg. In contrast, the policosanol content in wheat flour was reported to be less than 1 mg/kg of biomass, whereas wheat straw contained 164 mg/kg of biomass (*3*). Sugar cane peel contained approximately 270 mg/kg of biomass, while sugar cane leaves yielded 181 mg/kg of biomass. Perilla seeds have a total policosanol content of approximately 183 mg/kg of biomass, and the policosanol content of sorghum grains was between 880 and 1200 mg/kg (*4*). Clearly, switchgrass does not contain high policosanol concentrations similar to sorghum grains. However, from the results presented, the policosanol content of Oklahoma-grown August switchgrass was similar to that of perilla seeds (*4*). Using the average yield of Arkansas-grown switchgrass (10.14 Mg/ha) (*9*) and the maximum policosanol content (107.4 mg/kg), approximately 1.1 kg of policosanol/ha can be obtained.

From a compositional perspective, the total policosanols from switchgrass-extracted material were 0.4−2.1% C_{26}, 9.2−34% C_{28}, 35−68% C_{30}, and 42−80% C_{32} alcohols. For all extracted material, C_{26} was the least abundant policosanol. The composition of switchgrass-extracted policosanols was different from that of sorghum. Switchgrass-extracted policosanols contained approximately 10 times more C_{32} than sorghum-extracted policosanols. The presence of C_{32} alcohols has not been reported in perilla seeds or sugar cane. These results indicate that policosanol compositions may vary from species to species.

C_{28} is the main component of sugar-cane-extracted policosanols, and it was reported to lower LDL and increase HDL levels (*11*). C_{28} is possibly a mitigator in hypertension and high-cholesterol levels (*12*). It is possible that policosanol composition is important in conferring biological activity and that extracts rich in C_{32} have a different biological activity than those in C_{28}.

Although not within the scope of this work, researchers were intrigued by the presence of the peak at 14.9 min, as shown in **Figure 2**. Through a mass spectral library search (m/z 509) and through co-chromatographic experiments with authentic standards, the 14.9 min peak was identified as α-tocopherol. The content of α-tocopherol of Blackwell and Cave-in-Rock switchgrass varieties grown in the Arkansas and Oklahoma locations is shown in **Table 2**. The α-tocopherol extracted from both switchgrass varieties varied between 99 ± 28 and 441 ± 55 mg/kg in the months of September and October at both locations. Using the average α-tocopherol concentration of switchgrass, 4.4 kg of α-tocopherol could be obtained from 10.14 mg/ha of switchgrass. In both varieties and locations, the α-tocopherol content of December samples was significantly lower than those of September and October. The rapid decrease ($p < 0.0001$) in α-tocopherol in Oklahoma and Arkansas samples during November and December can most likely be attributed to frost events that eventually led to cell apoptosis. In Fayetteville, killing frosts occurred on the nights of the 2nd and 3rd November of 2006, where the temperature dropped to −3 °C.

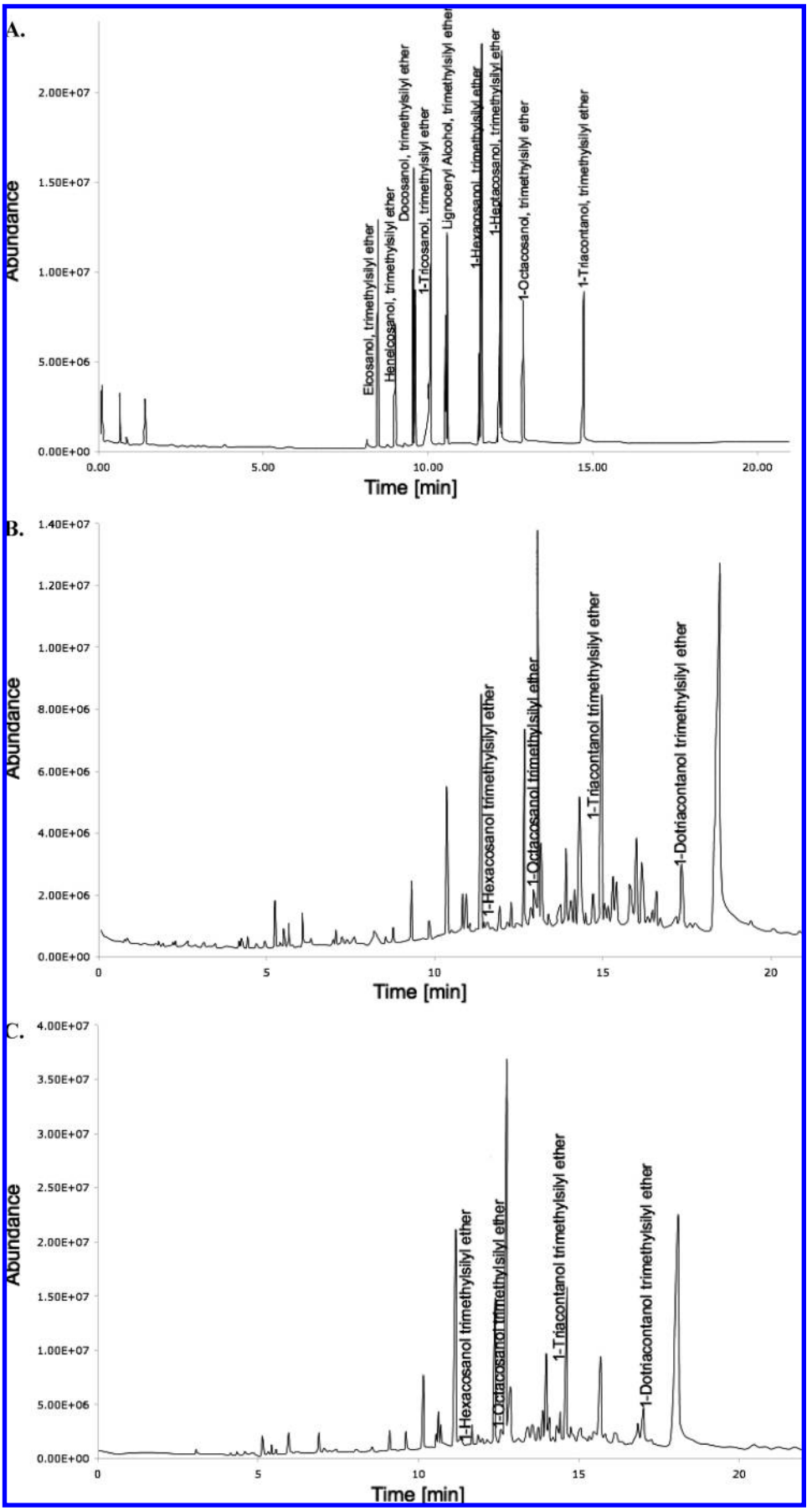

Figure 2. GC−MS chromatograms of policosanols: (A) standard policosanol reference compounds, (B) AR location Cave-in-Rock switchgrass sample, and (C) OK location Cave-in-Rock switchgrass sample. The retention times for hexacosanol, octacosanol, α-tocopherol, triacontanol, and dotriacontanol were 13.8, 14.8, 14.9, 16.2, and 18.2 min, respectively.

Table 1. Concentrations of Individual and Total Policosanols in Arkansas and Oklahoma Cave-in-Rock (CIR) and Blackwell (BW) Switchgrass Samples (mg/kg)[a]

			July	August	September	October	November	December
AR	CIR	C26	0.5 ± 0.1	0.6 ± 0.3	0.5 ± 0.1	0.8 ± 0.2	0.6 ± 0.3	0.7 ± 0.3
		C28	9.5 ± 2.9	10.7 ± 5.7	11.3 ± 3.3	13.9 ± 2.6	9.9 ± 4.1	11.7 ± 4.3
		C30	37.5 ± 4.2	37.5 ± 6.7	39.5 ± 2.6	44.4 ± 4.4	38.8 ± 4.4	41.9 ± 6.7
		C32	41.9 ± 4.2	42.5 ± 6.6	43.7 ± 2.9	48.3 ± 5.7	43.4 ± 6.0	47.5 ± 7.1
		total	89.5 ± 11.4	91.4 ± 19.4	95.0 ± 8.9	107.4 ± 12.9	92.7 ± 14.9	101.9 ± 18.5
	BW	C26	0.4 ± 0.3	0.6 ± 0.1	0.6 ± 0.4	0.7 ± 0.3	0.6 ± 0.3	0.7 ± 0.1
		C28	9.2 ± 3.7	10.1 ± 3.2	10.6 ± 1.5	10.7 ± 3.6	9.8 ± 5.0	12.7 ± 2.8
		C30	35.2 ± 5.1	37.7 ± 5.2	36.1 ± 1.9	36.9 ± 4.6	36.6 ± 6.5	40.9 ± 3.1
		C32	45.1 ± 8.6	44.7 ± 6.8	42.5 ± 2.5	45.1 ± 7.5	42.7 ± 7.6	51.3 ± 3.7
		total	89.9 ± 17.7	93.1 ± 15.4	89.7 ± 6.0	93.4 ± 16.0	89.7 ± 19.5	105.6 ± 9.6
OK	CIR	C26	1.6 ± 0.8	1.8 ± 0.2	1.6 ± 0.4	1.6 ± 0.5	1.2 ± 0.3	0.4 ± 0.2
		C28	25.7 ± 11.1	33.9 ± 5.5	27.5 ± 7.5	31.4 ± 7.2	22.7 ± 5.1	10.6 ± 6.6
		C30	53.3 ± 10.0	67.4 ± 3.7	65.7 ± 4.2	67.7 ± 8.9	64.3 ± 8.8	41.3 ± 8.3
		C32	65.2 ± 10.6	78.9 ± 5.0	75.5 ± 4.4	79.5 ± 5.6	75.4 ± 1.9	52.3 ± 9.6
		total	145.9 ± 32.7	182.0 ± 14.5	170.4 ± 16.5	180.2 ± 22.1	163.7 ± 16.3	104.7 ± 24.9
	BW	C26	1.5 ± 0.9	1.6 ± 0.6	2.1 ± 0.9	1.1 ± 0.1	0.8 ± 0.1	0.4 ± 0.3
		C28	25.3 ± 13.0	24.9 ± 9.5	32.9 ± 9.1	20.2 ± 4.4	17.5 ± 3.3	10.7 ± 6.2
		C30	54.6 ± 13.9	54.3 ± 11.6	59.3 ± 9.1	52.1 ± 2.8	49.7 ± 3.2	42.4 ± 7.1
		C32	71.0 ± 12.7	73.3 ± 10.3	78.0 ± 6.1	75.2 ± 4.5	62.4 ± 4.4	52.3 ± 0.5
		total	152.5 ± 40.5	154.3 ± 32.1	172.4 ± 25.1	148.7 ± 11.9	130.5 ± 11.1	105.8 ± 14.0

[a] The value after the ± represents the standard deviation.

Table 2. Concentrations of α-tocopherol in mg/kg for Arkansas and Oklahoma Cave-in-Rock and Blackwell Samples[a]

		July	August	September	October	November	December
AR	CIR	159.7 ± 34.8	258.0 ± 51.3	328.3 ± 91.8	441.1 ± 55.0	32.2 ± 7.9	9.2 ± 3.1
	BW	174.6 ± 50.2	232.6 ± 46.4	260.7 ± 65.3	286.7 ± 127.8	24.3 ± 13.7	11.1 ± 6.8
OK	CIR	557.3 ± 244.3	306.4 ± 45.0	311.8 ± 108.0	220.6 ± 107.1	60.2 ± 15.2	9.5 ± 5.4
	BW	334.2 ± 81.9	194.6 ± 29.6	252.1 ± 70.9	99.4 ± 28.7	20.2 ± 7.0	5.4 ± 1.0

[a] The value after the ± represents the standard deviation.

Vitamin E, in general, is a powerful antioxidant and consists of a group of eight isomers, four tocopherols (α-, β-, γ-, and δ-tocopherol), and four tocotrienols (α-, β-, γ-, and δ-tocotrienol). Tocopherols are produced by photosynthetic organisms, including all plants, algae, and most cyanobacteria. α-Tocopherol is present in leaf chloroplast (*13*) and is present in oilseed crops, such as soybean (*Glycine max*) and vegetable oil. α-Tocopherol is present in the leaves of various plants, such as spinach, parsley (*Petroselinum crispum*), tobacco (*Nicotiana tabacum*), and lettuce (*Lactuca sativa*). Barley (*Hordeum vulgare*), maize (*Zea mays*), pea (*Pisum sativum*), and wheat (*Triticum aestivum*) have α-tocopherol contents of 13, 5.6, 6.5, and 14 mg/kg dry weight, respectively (*14*). A report on the α-tocopherol content of barley showed that leaves contained 15.7 mg/kg fresh weight. By assuming that a leaf contains approximately 90% moisture (fresh weight), barley leaves would contain 157 mg/kg dry weight, which falls within the concentrations reported in this work. It is important to note that the time of the season that the α-tocopherol concentration of barley was measured was not specified and could lead to differences in overall content (*14*).

In conclusion, this work has shown that policosanols and α-tocopherol are present in switchgrass. Currently, sugar cane and beeswax are sources of policosanols. Switchgrass may present an alternative policosanol source, especially if further research shows that policosanol composition is of importance. Preliminary calculations show that 1.1 kg of policosanols and 4.4 kg of α-tocopherol could be obtained from 1 ha. The commercial value of these specialty chemicals will determine whether it is feasible to extract them within the framework of a biorefinery.

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