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Policosanol Contents and Compositions of Wheat Varieties

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Policosanol (PC) is the common name for a mixture of high molecular weight (20−36 carbon) aliphatic primary alcohols, which are constituents of plant epicuticular waxes. Wheat germ oil has been reported to improve human physical fitness, and this effect is attributed to its high PC, specifically its high octacosanol (OC) content. Although the PC composition of wheat leaves has been studied extensively, information on PC content and composition of wheat grain fractions is scarce. The objective of this study was to examine the PC contents and compositions of wheat grain fractions of 31 varieties grown in Oklahoma. PC compositions of the samples were identified using a gas chromatograph coupled with a mass spectrometer. The PC content of wheat bran was higher than that of the germ, shorts, and flour. The Trego and Intrada varieties had the highest PC content among the 31 wheat varieties studied. Tetracosanol (C24), hexacosanol (C26), and OC (C28) were the major PC components in all varieties. This study showed that wheat varieties grown under identical growing conditions and management differ significantly in PC content and composition.

KEYWORDS: Octacosanol; plant wax; policosanol; wheat

INTRODUCTION

Wheat (*Triticum aestium*) is among the most extensively grown crops in the world. It is believed that the first type of wheat cultivated was einkorn (*1*). Today, new varieties are usually bred for disease resistance, yield performance, or a set of quality characteristics. The agronomic research goals of the wheat improvement team at Oklahoma State University (OSU) are to improve leaf rust, stripe rust, soil-borne mosaic virus, aphid resistance, and tolerance to low-pH and Al-toxic soils. The variety selection at OSU has long been performed under a grain-only management system, but resources are being re-channeled toward selection in a dual-purpose environment under the GRAZEnGRAIN breeding system (*2*). The wheat samples used in this study were obtained from OSU wheat variety selection trial plots.

In plants, the surfaces that are exposed to the atmosphere usually have a layer that contains wax. Stems, fruits, petals, and leaves may all be covered with wax. The wax preserves the water balance of the plant, may minimize mechanical damage to the cells, and inhibits fungal and insect attacks. Leaf waxes have received the most attention. A number of research studies has been carried out on wheat leaf wax (*3*−*9*). The isolation of *n*-octacosanol (OC) from wheat wax was first reported by Pollard in 1933 (*3*). Later, Tulloch examined the OC composition in durum, club, and *Triticum aestivum* wheat leaf wax (*6*, *7*). The principal component of the wax from blades of young wheat was identified as a long-chain alcohol, OC. A study on *Triticum* and related species showed that alkanes (5−10%), esters (10−30%), aldehydes (≤5%), alcohols (15−55%), and acids (≤5%) were present in epicuticular wax of the plants examined (*10*).

Wheat germ oil (WGO) has been reported to improve human physical fitness, and this effect is attributed to its high policosanol (PC) content, specifically its high OC content (*11*, *12*). PC is a mixture of high molecular weight aliphatic primary alcohols, which are constituents of plant wax. There are numerous research studies indicating that 5−20 mg/day PC consumption is effective in lowering total cholesterol by 17−21% and low-density lipoprotein (LDL) by 21−29% and increasing high-density lipoprotein (HDL) by 8−15% (*13*−*16*). This is accomplished both by inhibiting cholesterol synthesis and by increasing LDL processing. There is also scientific evidence that PC has additional beneficial effects on smooth muscle cell proliferation, reducing platelet aggregation and LDL peroxidation (*15*, *17*−*19*). PC formulations are also being used as antifatigue drugs (*20*). One study carried out with tail-suspended rats indicated that OC could counteract some effects of simulated weightlessness on rats, suggesting that OC enriched foods might benefit astronauts during space travels (*21*).

In a previous study, we have compared the PC content and composition of wheat, sugar cane, and beeswax (*22*). However, to the best of our knowledge, there are no published data on variations in PC content and composition of wheat grain by variety. Hence, the objective of this study was to examine PC content and composition of wheat varieties developed for the Oklahoma region.

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Table 1. Policosonal Content (mg/kg)[a] and Composition of Wheat Grain Fractions

PC[b]/grain fraction	C20	C21	C22	C23	C24	C26	C27	C28	C30	total PC
germ	n.d.	n.d.	$2.8^{a} \pm 0.5$	n.d.	$1.4^{a} \pm 0.2$	n.d.	$0.52^{a} \pm 0.09$	$2.9^{a} \pm 0.3$	$2.5^{a} \pm 0.3$	$10.1^{a} \pm 0.7$
bran	$3.78^{a} \pm 0.02$	$1.72^{a} \pm 0.03$	$2.73^{a} \pm 0.02$	1.80 ± 0.03	$10.68^{b} \pm 0.01$	$4.87^{a} \pm 0.03$	n.d.	$4.39^{b} \pm 0.02$	n.d.	$29.97^{b} \pm 0.06$
shorts	$0.72^{b} \pm 0.06$	$0.29^{b} \pm 0.02$	$0.21^{b} \pm 0.02$	n.d.	$0.82^{c} \pm 0.01$	$0.45^{b} \pm 0.03$	$0.19^{b} \pm 0.02$	$0.39^{c} \pm 0.01$	$0.22^{b} \pm 0.03$	$3.29^{c} \pm 0.08$
flour (Kalvesta)	n.d.[c]	n.d.	n.d.	n.d.	n.d.	n.d.	$0.17^{b} \pm 0.01$	n.d.	n.d.	$0.17^{d} \pm 0.01$

[a] Means in the same column with the same letter are not significantly different at $p > 0.05$. [b] Eicosanol (C20), heneicosanol (C21), docosanol (C22), tricosanol (C23), tetracosanol (C24), hexacosanol (C26), heptacosanol (C27), octacosanol (C28), and triacontanol (C30). [c] n.d.: not detected.

Table 2. Policosanol Content (mg/kg) and Composition of Oklahoma Grown Wheat Varieties

PC[a] variety	C20	C21	C22	C23	C24	C26	C27	C28	C30	total PC
Cossack	2.4 ± 0.2	0.62 ± 0.06	1.8 ± 0.2	1.3 ± 0.2	6.4 ± 0.9	3.6 ± 0.8	0.57 ± 0.05	2.4 ± 0.3	0.95 ± 0.09	20 ± 1
Chisholm	2.37 ± 0.06	0.78 ± 0.06	2.4 ± 0.4	1.5 ± 0.4	6.4 ± 1.3	4.8 ± 1.2	0.6 ± 0.2	1.9 ± 0.2	1.1 ± 0.3	21.9 ± 1.9
AP502CL	2.1 ± 0.4	1.44 ± 0.07	3.1 ± 0.3	2.13 ± 0.09	7.4 ± 0.9	5.6 ± 0.2	1.04 ± 0.02	2.7 ± 0.4	1.20 ± 0.07	26.7 ± 1.1
Venango	0.87 ± 0.04	0.42 ± 0.04	0.8 ± 0.1	0.51 ± 0.09	1.81 ± 0.05	1.2 ± 0.3	0.25 ± 0.02	0.8 ± 0.1	0.3 ± 0.1	7.0 ± 0.4
OK102 WT3P1	0.49 ± 0.08	n.d.	0.3 ± 0.1	n.d.	0.80 ± 0.09	1.0 ± 0.2	n.d.	0.4 ± 0.1	n.d.	3.0 ± 0.3
Cisco	1.29 ± 0.03	0.65 ± 0.09	1.94 ± 0.09	1.12 ± 0.09	6.8 ± 0.5	3.8 ± 0.2	0.98 ± 0.06	1.9 ± 0.2	n.d.	18.5 ± 0.6
Coronado	1.3 ± 0.2	n.d.	2.1 ± 0.2	n.d.	3.7 ± 0.3	2.5 ± 0.3	n.d.	1.3 ± 0.3	n.d.	10.9 ± 0.6
OK94P549-11	1.7 ± 0.4	0.65 ± 0.05	1.3 ± 0.3	1.00 ± 0.07	4.6 ± 0.2	3.1 ± 0.1	n.d.	1.50 ± 0.02	n.d.	13.9 ± 0.6
Kalvesta	0.89 ± 0.09	n.d.	1.2 ± 0.1	n.d.	2.2 ± 0.1	1.6 ± 0.1	n.d.	0.71 ± 0.08	n.d.	6.6 ± 0.2
Triumph 64	0.84 ± 0.06	n.d.	2.0 ± 0.2	0.72 ± 0.08	2.8 ± 0.3	1.8 ± 0.1	n.d.	0.7 ± 0.2	n.d.	8.9 ± 0.4
Dumas	0.67 ± 0.06	n.d.	1.4 ± 0.2	n.d.	1.9 ± 0.3	1.3 ± 0.2	n.d.	0.70 ± 0.06	n.d.	6.0 ± 0.4
OK102	0.74 ± 0.09	n.d.	0.7 ± 0.2	n.d.	1.4 ± 0.2	1.03 ± 0.08	n.d.	0.75 ± 0.05	n.d.	4.6 ± 0.3
2145	1.66 ± 0.01	0.9 ± 0.1	3.12 ± 0.09	2.17 ± 0.08	6.9 ± 0.4	4.3 ± 0.1	0.75 ± 0.02	3.0 ± 0.3	n.d.	22.8 ± 0.5
Cutter	1.90 ± 0.06	0.68 ± 0.05	1.73 ± 0.03	0.75 ± 0.05	6.2 ± 0.1	4.1 ± 0.1	0.65 ± 0.04	4.0 ± 0.6	n.d.	20.0 ± 0.6
Above	1.40 ± 0.06	0.7 ± 0.1	2.4 ± 0.1	1.09 ± 0.09	5.1 ± 0.4	3.8 ± 0.2	1.03 ± 0.02	1.99 ± 0.09	n.d.	17.5 ± 0.5
Tam 110	1.5 ± 0.2	0.53 ± 0.09	2.2 ± 0.2	0.91 ± 0.07	4.7 ± 0.6	3.9 ± 0.7	n.d.	2.2 ± 0.3	n.d.	15.9 ± 1.0
Tam 302	1.87 ± 0.09	0.7 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	4.9 ± 0.5	2.2 ± 0.4	n.d.	1.85 ± 0.08	n.d.	13.8 ± 0.7
Enhancer	2.4 ± 0.3	0.91 ± 0.07	1.5 ± 0.2	1.62 ± 0.03	7.0 ± 0.4	4.7 ± 0.4	n.d.	2.4 ± 0.2	n.d.	20.5 ± 0.7
2137	1.2 ± 0.2	0.53 ± 0.03	1.2 ± 0.1	n.d.	3.4 ± 0.5	2.0 ± 0.3	n.d.	1.2 ± 0.2	n.d.	9.5 ± 0.7
Tam 111	1.08 ± 0.07	0.58 ± 0.04	1.4 ± 0.2	1.0 ± 0.1	4.7 ± 0.2	4.0 ± 0.6	n.d.	1.3 ± 0.1	n.d.	14.1 ± 0.7
Jagger	1.4 ± 0.3	0.62 ± 0.04	1.22 ± 0.02	n.d.	3.78 ± 0.05	2.45 ± 0.09	n.d.	1.33 ± 0.08	n.d.	10.8 ± 0.3
Lakin	1.0 ± 0.1	0.50 ± 0.08	0.95 ± 0.07	n.d.	3.8 ± 0.3	2.6 ± 0.2	n.d.	0.85 ± 0.08	n.d.	9.7 ± 0.4
Avalanche	0.71 ± 0.05	n.d.	1.5 ± 0.1	n.d.	3.4 ± 0.1	2.0 ± 0.1	n.d.	0.79 ± 0.08	n.d.	8.4 ± 0.2
Custer	0.67 ± 0.09	n.d.	1.1 ± 0.1	0.49 ± 0.05	6.2 ± 0.2	4.1 ± 0.3	n.d.	0.86 ± 0.003	n.d.	13.4 ± 0.4
Jagalene	1.29 ± 0.08	n.d.	1.74 ± 0.08	n.d.	3.7 ± 0.2	2.8 ± 0.2	n.d.	0.91 ± 0.04	n.d.	10.4 ± 0.3
Thunderbolt	0.93 ± 0.09	n.d.	1.1 ± 0.1	n.d.	4.7 ± 0.3	3.7 ± 0.2	n.d.	0.96 ± 0.06	n.d.	11.4 ± 0.4
2174	0.98 ± 0.06	n.d.	1.05 ± 0.09	n.d.	2.09 ± 0.08	2.9 ± 0.3	n.d.	0.90 ± 0.006	n.d.	8.0 ± 0.3
Intrada	3.3 ± 0.1	1.2 ± 0.1	3.0 ± 0.1	1.00 ± 0.07	5.3 ± 0.2	7.2 ± 0.3	1.3 ± 0.1	7.7 ± 0.2	7.0 ± 0.2	37.0 ± 0.5
G1878	3.3 ± 0.1	0.97 ± 0.05	3.00 ± 0.09	0.71 ± 0.06	3.5 ± 0.1	5.26 ± 0.07	1.14 ± 0.07	5.0 ± 0.1	n.d.	22.9 ± 0.2
OK 101	3.5 ± 0.2	1.03 ± 0.07	2.32 ± 0.07	0.72 ± 0.06	4.9 ± 0.2	4.2 ± 0.1	0.72 ± 0.06	3.42 ± 0.09	1.0 ± 0.2	21.8 ± 0.4
Trego	4.9 ± 0.2	n.d.	6.8 ± 0.2	1.9 ± 0.2	13.8 ± 0.4	13.1 ± 0.4	2.38 ± 0.07	8.9 ± 0.3	4.4 ± 1.0	56.2 ± 1.2

[a] See **Table 1** for abbreviations.

MATERIALS AND METHODS

Materials. Hard red winter and hard white winter wheat varieties examined in this study were grown at the Oklahoma State University, Stillwater Agronomy Research Station under identical growing conditions and management. The hard red winter wheat varieties studied were Cossack, Chisholm, AP502CL, Venango, OK102 WT3P1, Cisco, Coronado, OK94P549-11 (Endurance), Kalvesta, 2137, Tam 111, Jagger, Custer, Jagalene, Thunderbolt, 2174, Triumph 64, Dumas, OK102, 2145, Cutter, Above, TAM 110, TAM 302, and Enhancer. Avalanche, Intrada, Trego, and Lakin were the hard white winter wheat varieties examined in this study. Further information on agronomic properties of these varieties can be found on the OSU Wheat Improvement Team web site (*2*).

The samples of wheat grain fractions (germ, bran, and shorts, **Table 1**) were byproducts of a commercial milling operation (ADM Milling Corp., Enid, OK). These samples consisted of a mixture of wheat varieties grown in Kansas and Oklahoma. The grain samples from wheat varieties grown in Oklahoma (**Table 2**) were milled at ADM Milling Corp. (Enid, OK) using a Buhler pilot mill (Buhler, Switzerland) to separate bran and endosperm fractions. Each variety was milled separately, and the milling system was cleaned between varieties to avoid sample carryover. The whole grain samples were conditioned to 15.2% moisture before milling as moisture helps to prevent bran breaking and improves separation from floury endosperm. The grain fractions obtained from milling were analyzed for their PC content and composition. The individual PC standards used for peak identification, eicosanol (C20), heneicosanol (C21), docosanol (C22), tricosanol (C23), tetracosanol (C24), hexacosanol (C26), heptacosanol (C27), and octacosanol (C28) were purchased from Sigma (Sigma-Aldrich Corporation, St. Louis, MO) and used without further purification (97% and higher purity). Triacontanol (C30) (96%) was obtained from Aldrich (Sigma-Aldrich Corporation, St. Louis, MO). *N*-Methyl-*N*-(trimethlysilyl)-trifluoroacetamide (MSTFA) from Pierce (Rockford, IL) was used as the derivatization reagent. All other chemicals used in this study were reagent grade unless otherwise stated.

Analytical Procedure. Stock solutions of PC were prepared in chloroform (HPLC grade, Burdick and Jackson, Muskegon, MI) and derivatized with MSTFA at 60 °C for 15−20 min. The desired concentrations of standard solutions were prepared by dilution of the stock solutions.

Wheat samples were ground in a coffee grinder (Black and Decker CBG5, Miami Lakes, FL) at medium speed for 1 min. Ten grams of ground sample was hydrolyzed by refluxing with 100 mL of 1.0 N NaOH in methanol for 45 min. The mixture was cooled and filtered through glass wool using a glass funnel. Millipore water was added to the filtrate. Then, the solution was extracted with HPLC grade diethyl ether (Burdick and Jackson, Muskegon, MI). The extraction was repeated 3 times using equal volumes of diethyl ether. The ethyl ether phase collected from three extractions was combined and washed with Millipore water until the pH of the water phase reached 7. The ether

extract was evaporated to dryness under nitrogen at 40 °C using a Reacti-Vap evaporation unit (Model 18780, Pierce, Rockford, IL) after drying over anhydrous sodium sulfate (ACS grade, EMD Chemicals Inc., Gibbstown, NJ). The residue was transferred to a 1 mL volumetric flask, and 0.5 mL of chloroform and 250 μL of silylation reagent (MSTFA) were added. Then, the solution was heated at 60 °C for 15−20 min for derivatization. Chloroform was added to reach a total sample volume of 1 mL before analysis.

GC−MS Analysis. Trimethylsilyl derivatives of alcohols were analyzed using a HP 6890 Series Gas Chromatography (GC) system coupled with a 5973 Network mass selective detector (Agilent Technologies, Palo Alto, CA). A fused silica capillary Equity-5 (30 m × 0.25 mm × 0.5 μm film thickness) from Supelco was used for the analysis. The oven temperature was programmed from 150 to 320 °C with a 4 °C/min heating rate and maintained at 320 °C for 15 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was 300 °C. Mass spectrophotometer (MS) parameters were as follows: MS transfer line 280 °C, ion source 230 °C, and MS quadrupole temperature 150 °C. The ionization energy was 70 eV. The scan range and rate were 100−600 amu and 2 scans/s, respectively. A total of 1 μL of sample was injected into the GC−MS by an autosampler (HP 7683, HP Company, Wilmington, DE). The split ratio was 1:10. The data collection and analysis were managed using HP Chemstation (Enhanced Chemstation G1701 DA Version D.00.00.38, Agilent Technologies, Palo Alto, CA). The PC composition of the samples was identified by direct comparison of their chromatographic retention times and the mass spectra with those of the authentic compounds. The peaks were also confirmed with the NIST/EPA/NIH Mass Spectral Library (Version 2.0).

Statistical Analysis. All extraction runs and analyses were carried out at least in duplicate and in randomized order with the mean values being reported. Analysis of variance (ANOVA) of the results was performed using the General Linear Model procedure of SAS (Software Version 8.1. SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The analysis of commercially milled wheat grain fractions, germ, bran, shorts, and flour showed that PC was concentrated in the bran (**Table 1**). The PC content of the wheat flour was very low. Wheat germ contained a significant amount of PC (10.1 mg/kg). These results were expected since PC is associated with lipids and wax in plant tissues. Significant differences ($p < 0.05$) were also observed in PC composition of wheat grain fractions. About 36% of the total PC in the bran fraction was constituted of C24. C20, C26, and C28 were also present in significant amounts in the bran (>12% of total PC). There have been reports indicating that C28 is the main PC component in the germ (*3*, *23*−*25*). Some of these studies on wheat germ PC are quite old, dating back to the 1930s, and focus on OC rather than complete PC composition. The use of GC−MS in this study allowed us to identify individual PC components in a very complicated chromatogram. The overlapping peaks were resolved by the Extracted Ion Chromatograms tool on the Chemstation software. Consequently, a more accurate determination of PC composition of the samples was achieved. The major PC components in wheat germ were C22, C28, and C30, which constituted over 81% of the total PC present.

The study on variations in PC content of wheat varieties was based on the bran fraction of the grain because the results obtained from the analysis of grain fractions indicated that flour did not contain a significant amount of PC (**Table 1**). Furthermore, pilot scale equipment used for milling was not capable of separating bran and germ fractions. Hence, bran fraction for these samples (**Table 2**) consisted of both bran and germ. The total PC content of the varieties examined in this study varied significantly. OK 102 WT3P1 (3.0 mg/kg bran) and Trego (56.2 mg/kg bran) were the varieties with the lowest and highest PC content, respectively (**Table 2**). Intrada also contained significantly higher ($p < 0.05$) PC content than the other varieties. Both wheat samples with high PC content, Intrada and Trego, were hard white winter varieties. It is interesting to note that when extracts from three hard winter wheat varieties, Trego, Akron, and Platte, were evaluated for their potential to inhibit lipid peroxidation in fish oil, the Trego extract had the highest capacity to suppress lipid oxidation (*26*). Unfortunately, the chemical composition of the wheat extracts used for the study was not reported. The authors suggested that Trego extracts may have the potential to be food antioxidants. The effect of chemical composition including PC content and composition of wheat extracts on lipid oxidation requires further research. The high PC concentration of Trego also makes this variety a candidate as a source of PC for functional foods and nutraceutical applications.

Large variations were observed in the PC composition of wheat varieties examined in this study. In general, C24 and C26 were the most abundant PCs present in bran fractions of all Oklahoma grown wheat varieties. Total C24 + C26 contents of Custer and Thunderbolt were very high (about 77 and 74% of total PC, respectively) as compared to that of the other varieties. Intrada and Trego contained large amount of C28, 21 and 16% of the total PC, respectively.

This study showed that PC was concentrated in the bran fraction of wheat grain. It was found that significant variations existed in both PC content and composition among wheat varieties. Intrada and Trego were two varieties that had the highest PC content. C24 and C26 were the major components of PC in all wheat varieties. The Intrada and Trego varieties also contained a very high amount of C28, indicating that these varieties may be potential PC sources for functional foods and nutraceutical applications.

The study of wheat surface waxes is also important for agronomic reasons because of the significance of these compounds in water loss, agricultural spray efficiency, and mechanical damage. It is well-documented that mutations affect the chemical composition of epicuticular waxes of several plant families (*27*). A study on the chemical genetics of PC formation in wheat varieties requires examination of biosynthetic pathways governing the formation of precursors of all the wax classes. This study is a preliminary step toward the understanding of the biosynthesis of wax components in wheat.

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