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Policosanol Content and Composition in Perilla Seeds

PRAKASH ADHIKARI, KEUM TAEK HWANG,* JAE NAM PARK, AND CHOONG KI KIM

Department of Food Science and Human Nutrition, and Center for Healthcare Technology Development, Chonbuk National University, Jeonju 561-756, Korea

Policosanols, long-chain alcohols, have many beneficial physiological activities. Contents and compositions in perilla seeds *(Perilla frutescens)* produced in Korea and China were determined. Waxy materials were extracted from perilla seeds using hot hexane. Yield of the waxy materials from perilla seeds was 72.1 mg/100 g of dry weight. Contents and compositions of the waxy materials and policosanols were identified and quantified by TLC, HPLC, and GC. Major components of the waxy materials from Korean and Chinese perilla seeds were policosanols (25.5 and 34.8%, respectively), hydrocarbons (18.8 and 10.5%), wax esters, steryl esters and aldehydes (53.0 and 49.8%), acids (1.7 and 2.1%), and triacylglycerols (1.0 and 2.9%), determined by HPLC. For comparison, waxy materials of sesame seeds were also analyzed. Yield of the waxy materials from sesame seeds were 8.6 mg/100 g. Less than 5% policosanols were detected in the waxy materials extracted from sesame seeds produced in Korea and China. Wax esters or steryl esters accounted for 93−95% of the sesame waxy materials. Policosanols in the perilla seeds were composed of 67−68% octacosanol, 16−17% hexacosanol, 6−9% triacontanol, and others.

KEYWORDS: Octacosanol; perilla seeds; policosanols; sesame seeds; wax

INTRODUCTION

Policosanols are a mixture of long-chained primary alcohols (*1*). Commercially, these are available as nutritional supplements with hexacosanol (26:0), octacosanol (28:0), triacontanol (30:0), and dotriacontanol (32:0) (*2*). They have various beneficial physiological activities such as reducing platelet aggregation, endothelial damage, and foam cell formation (*3, 4*), increasing muscle endurance (*5*), and improving exercise performance of coronary heart disease patients (*6*). They have also been reported to reduce LDL cholesterol levels and increase HDL cholesterol levels in blood (*7*). Octacosanol, one of the most abundant alcohols in policosanols, is taken as an alternative to aspirin for patients suffering from gastric irritation due to its cytoprotective effects (*8*).

Composition of policosanols extracted from waxy materials of different sources such as beeswax, rice bran, wheat germ, sugar cane, and grain sorghum has been identified and analyzed using different chromatographic techniques (*9, 10*). Among them, grain sorghum (unpolished) is reported to have the highest yield of waxy materials (223 mg/100 g of dry weight) and high content of policosanols in free, nonesterified forms (*2*). HPLC analysis of waxy materials from grain sorghum showed that aldehydes (46−55%, w/w), policosanols (37−41%), and acids (4−7%) were the major components (*10*−*12*). Policosanols extracted from the waxy materials of grain sorghum kernels were composed of 6−8% hexacosanol, 43−47% octacosanol, 40−43% triacontanol, and other alcohols, analyzed by GC (*10*).

Perilla (*Perilla frutescens*) seeds are a traditional source of oils produced in Korea, India, China, and other Asian countries. Annual production of perilla seeds in Korea is about 40000 metric tons and expelled perilla oil production is the third largest among edible oils in Korea (*13*). Perilla seed oil contains considerably high levels of linolenic acid (*14*). Despite its nutritional benefits, perilla seeds are largely unknown in Western society. Therefore, the extraction of oils and oil-based products from such plant seeds would provide a new supplement in the world market.

To our knowledge, a comprehensive study regarding policosanol levels for perilla seeds from various sources has not been reported. The objective of this study was to determine the contents and compositions of waxy materials and policosanols in perilla seeds. For comparison, sesame seeds were also analyzed for waxy materials and policosanol contents and composition.

MATERIALS AND METHODS

Materials. Perilla (*Perilla frutescens*) and sesame (*Sesamum indicum*) seeds of Korean and Chinese origin were purchased from a traditional market in Jeonju, Korea. All the samples were analyzed on a dry weight basis.

Moisture and Crude Lipid Contents. Moisture content in perilla and sesame seeds was determined using a drying oven (F-600M; Jeio-Tech. Co., Seoul, Korea) set at 104 °C. Crude lipids were extracted via Soxhlet extraction for 14 h using diethyl ether as a solvent. Three

* Corresponding author. Tel.: +82-63-270-3857. Fax: +82-63-270-3854. E-mail: keum@chonbuk.ac.kr.

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grams of crushed perilla and sesame seeds were used for each determination.

Extraction of Waxy Materials. Perilla and sesame seeds (800 g) were washed with tap water and placed on an absorbent towel. Immature seeds were removed by hand. Remaining seeds were dried at room temperature for 24 h. The uncrushed seeds were refluxed with 800 mL of hexane for 30 min in a round-bottom flask (2 L) at 70 °C. The warm mixture of seeds and hexane was filtered through a coffee filter paper to remove seeds. The filtrate was then passed through Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, UK) to remove impurities and stored at −18 °C for 8 h to precipitate the waxy materials. The filtrate was refiltered through Whatman No. 42 filter paper. Precipitate on the No. 42 filter paper was vacuum-dried at room temperature and collected as waxy materials.

TLC of Waxy Materials. TLC analysis of waxy materials extracted from the seeds was performed according to the previous report (*11*). Waxy material solution in hexane (about 10 μg/10 μL) was spotted on a 20 × 20 cm, silica gel 60, 250 μm TLC plate (EM Science, Darmstadt, Germany). The developing solvent was a mixture of hexane, diethyl ether, and acetic acid (85:15:2, v/v/v). Developed bands were visualized by dipping the plate in 10% cupric sulfate solution containing 8% phosphoric acid for 5 s. Then the TLC plate was dried for 5 min and kept in an oven at 150 °C until the developed bands were charred.

Compositional Analysis of Waxy Materials. Components were determined according to the previous report (*12*), using an HPLC equipped with a 250 mm × 4.6 mm i.d., 5 μm Luna silica column (Phenomenex, Torrance, CA) connected with a 4 × 3 mm i.d. guard column (Phenomenex). The detector was an Alltech Evaporative Light Scattering Detector 800 (Deerfield, IL), operated at 40 °C with nitrogen pressure of 3.5 bar. Two Waters 510 HPLC pumps (Waters Corp., Milford, MA) were operated in gradient mode at a flow rate of 1 mL/min. Elution solvent consisted of a gradient of hexane (solvent A) and 0.2% acetic acid in methyl *tert*-butyl ether (solvent B), with the following profile: 0−2 min, 100% A; 3−10 min, 95% A; 14 min, 55% A; 23−26 min, 0% A; and 27−40 min, 100% A. The column and guard column were heated to 38−40 °C using a Waters Column Heater Module. Exposed lines from injection loop to detector connection were maintained at 38−40 °C wrapped with a heating tape. Samples were prepared in hexane (2 μg/20 μL), and 20 μL of each sample was injected for the analysis.

Compositional Analysis of Policosanols Fractionated from Waxy Materials. The composition of policosanols in the waxy materials was analyzed using GC according to the previous method (*10*). The policosanol fraction (2 mL) collected from HPLC (20 μg of waxy materials) was derivatized to trimethylsilyl (TMS) ethers (10 min at 60 °C) using 0.05 mL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (Sigma Chemical, St. Louis, MO) and 0.2 mL of chloroform. A standard solution of alcohols (docosanol, tricosanol, and tetracosanol (Nu-Chek Prep, Inc., Elysian, MN) and hexacosanol, heptacosanol, octacosanol, and triacontanol (Sigma Chemical, St. Louis, MO)) was prepared in 0.2 mL of chloroform (1−8 μg of each) and derivatized as above for the identification of retention times and the calculation of their response factors. The TMS ether derivatives (2 μL) were injected into a 6980 Series GC (Hewlett-Packard, Wilmington, DE) equipped with a 30 m × 0.25 mm i.d., 0.25 μm, DB-5 column (J&W Scientific, Folsom, CA), flame-ionization detector (FID), and helium as a carrier gas. Injector and detector temperatures were both set at 315 °C. The oven was programmed to start and hold at 150 °C for 1 min before increasing to 210 °C at 20 °C/min, increasing to 310 °C at 4 °C/min, holding at 310 °C for 1 min, increasing to 315 °C at 25 °C/min, and finally holding for 5 min.

RESULTS AND DISCUSSION

Moisture and lipid contents of perilla seeds of Korean and Chinese origin are shown in **Table 1**. Waxy materials extracted from the perilla seeds of both origins were about 72 mg/100 g (**Table 1**). Waxy materials from the sesame seeds was much less (8 mg/100 g) than those from the perilla seeds. Grain sorghum is known to contain more (223 mg/100 g) waxy materials as compared to the perilla seeds (*2*). Waxy materials from brown rice (32.9 mg/100 g) and purple rice (61.2 mg/100 g) are less than those from the perilla seeds (*2*).

Table 1. Mean Values of Moisture, Crude Lipids, and Waxy Materials in Perilla and Sesame Seeds[a]

sample	moisture (%, w/w)	crude lipids (%, dry weight)	waxy materials (mg/100 g of dry weight)
perilla seeds (Korean)	5.6 ± 0.01	51.2 ± 0.10	72.1 ± 0.01
perilla seeds (Chinese)	8.2 ± 0.33	48.4 ± 0.47	72.0 ± 0.03
sesame seeds (Korean)	5.8 ± 0.15	42.4 ± 0.43	8.3 ± 0.02
sesame seeds (Chinese)	5.9 ± 0.21	45.9 ± 0.70	8.6 ± 0.01

[a] Data are means ±S.D. ($n = 3$).

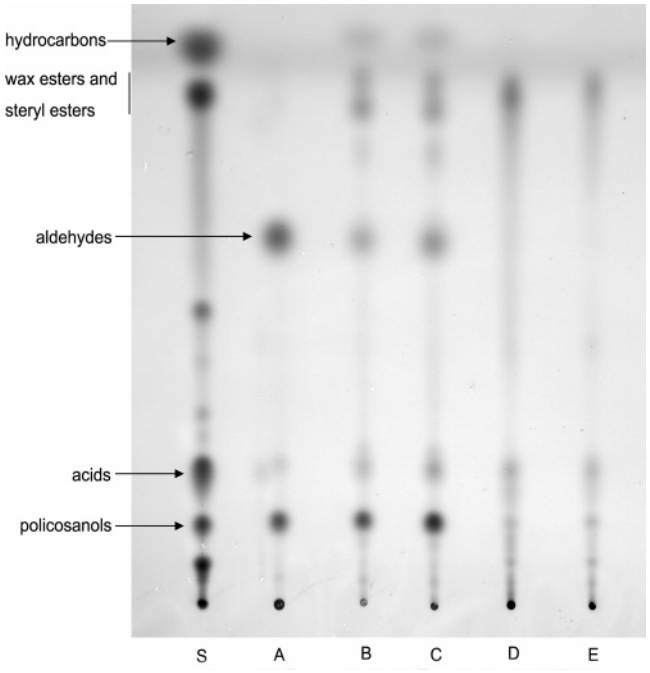

Figure 1. Thin-layer chromatography of waxy materials extracted from grain sorghum, perilla, and sesame seeds using a 20 × 20 cm, silica gel 60, 250 μm TLC plate and a developing solvent of hexane, diethyl ether, and acetic acid (85:15:2; v/v/v). S, standard mixture of octacosane, tetracosanoyl tetracosanoate, tetracosanoic acid, cholesterol, and octacosanol; A, grain sorghum; B, perilla seeds (Korean); C, perilla seeds (Chinese); D, sesame seeds (Korean); and E, sesame seeds (Chinese).

Three dark and two light spots were detected on the TLC plate for the waxy materials of perilla seeds (**Figure 1**). The spot for policosanols was relatively intense. Compositions of the waxy materials from perilla seeds were similar to those from grain sorghum except that the waxy materials from perilla seeds contained more hydrocarbons and wax/steryl esters than those from grain sorghum. Most of the waxy materials from sesame seeds might be wax esters since a pink color did not appear on the TLC plate during charring (steryl esters turn pink during charring on TLC).

The major components in the waxy materials from perilla seeds of Korean and Chinese origin were policosanols (25.5 and 34.8%, respectively), wax esters, steryl esters, and aldehydes (53.0 and 49.8%), hydrocarbons (18.8 and 10.5%), acids (1.7 and 2.1%), and triacylglycerols (1.0 and 2.9%), analyzed by HPLC (**Table 2**, **Figure 2**). The contents of policosanols in the waxy materials from Korean and Chinese origin perilla seeds

Table 2. HPLC-Analyzed Composition of Waxy Materials Extracted from Perilla and Sesame Seeds (%, w/w)[a]

sample	alcohols	wax esters, steryl esters, and aldehydes	acids	triacylglycerols	hydrocarbons
perilla seeds (Korean)	25.5 ± 0.86	53.0 ± 1.50	1.7 ± 0.05	1.0 ± 1.42	18.8 ± 0.69
perilla seeds (Chinese)	34.8 ± 1.53	49.8 ± 4.68	2.1 ± 0.32	2.9 ± 2.66	10.5 ± 0.30
sesame seeds (Korean)	1.8 ± 0.04	95.1 ± 3.63	1.7 ± 0.29	1.0 ± 1.31	n.d.[b]
sesame seeds (Chinese)	1.9 ± 0.65	93.7 ± 0.18	1.8 ± 0.23	2.7 ± 3.74	n.d.

[a] Data are means ±S.D. ($n = 2$). [b] Not detected.

Table 3. Policosanol Compositions of Perilla Seeds, Analyzed by GC (%, w/w)[a]

sample	C24:0	C26:0	C27:0	C28:0	C29:0	C30:0
perilla seeds (Korean)	1.6 ± 0.13	17.6 ± 0.01	2.4 ± 0.01	68.4 ± 1.44	2.3 ± 0.07	6.8 ± 1.42
perilla seeds (Chinese)	1.5 ± 0.06	16.6 ± 0.43	2.4 ± 0.43	67.4 ± 0.07	2.5 ± 0.15	9.1 ± 0.17

[a] Data are means ± S.D. ($n = 3$).

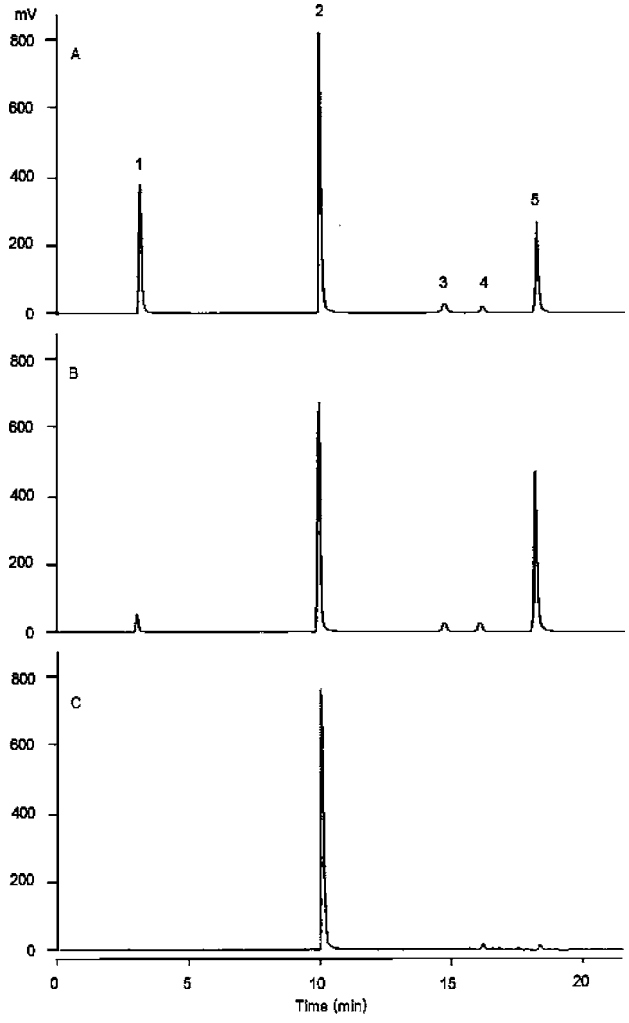

Figure 2. HPLC chromatograms of waxy materials extracted from perilla and sesame seeds. **A**, perilla seeds (Korean); **B**, perilla seeds (Chinese); and **C**, sesame seeds (Korean). (1) Hydrocarbons, (2) wax esters, steryl esters, and aldehydes, (3) triacylglycerols, (4) acids, and (5) policosanols.

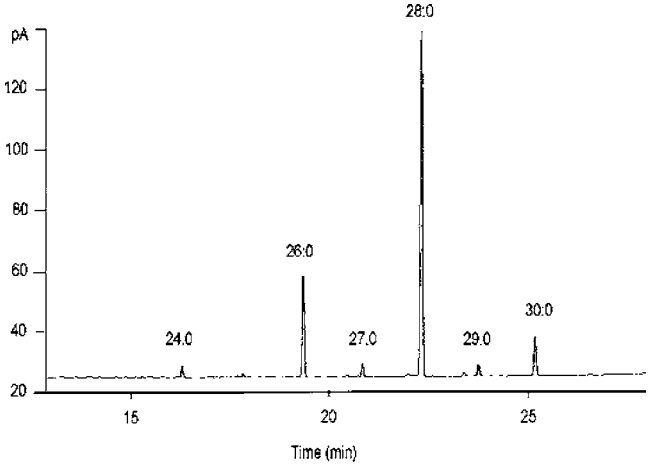

Figure 3. Gas chromatogram of policosanols in the waxy materials extracted from Korean perilla seeds.

were similar to those in the unpolished and polished grain sorghum (34−33% and 25−29%, respectively) (*2*).

Policosanols in the waxy materials from perilla seeds of both origins were composed of 66−67% octacosanol, 16−17% hexacosanol, 6−9% triacontanol, 2−3% heptacosanol, 1−3% nonacosanol, and 1−2% tetracosanol (**Table 3**, **Figure 3**). The octacosanol level in the policosanols from perilla seeds was somewhat higher than that from grain sorghum (unpolished and polished), which was about 46% (*2*).

Policosanols are traditionally produced by saponification of wax esters, the major forms of vegetable waxes. Policosanols in perilla seeds exist in free or nonesterified alcohols similar to those in grain sorghum. A saponification process for preparation of policosanols from perilla seeds is unnecessary and would avoid a step used in traditional policosanol preparation. This study indicates that consumption of perilla seeds may be beneficial in supplying policosanols in a diet since perilla seeds contain considerable amounts of policosanols compared to other major staple foods (*2*).

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