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Policosanol contents of beeswax, sugar cane and wheat extracts

Sibel Irmak, Nurhan Turgut Dunford *, Jeff Milligan

Oklahoma State University, Department of Plant and Soil Sciences, and Food and Agricultural Products Research and Technology Center, Room 103, Stillwater, OK 74078, USA

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Abstract

Policosanol (PC) is a mixture of high molecular weight aliphatic primary alcohols. Currently, a number of dietary supplements containing PC are commercially available in the US market. The majority of these products are prepared from beeswax or sugar cane extracts. The main objective of this study was to compare the PC contents and compositions of beeswax, sugar cane and wheat as PC sources. The PC contents and compositions of several commercial dietary supplements were also analyzed. The precipitate formed during the cold storage of commercially hexane-extracted wheat germ oil (WGO) contained the highest total PC (628 mg/kg) among the wheat extracts and milling products examined in this study. The total PC contents of wheat straw (164 mg/kg) and sugar cane peel (270 mg/kg) were of the same order of magnitude. The total PC contents of brown beeswax were about 20 and 45 times higher than those of the WGO-solids and sugar cane peel, respectively. Commercial dietary supplements contained less total PC than were claimed on the product labels. The PC compositions of the samples analyzed in this study varied significantly with the source. Wheat can be a viable PC source for further product development or health benefits.

Keywords: Beeswax; Dietary supplement; Policosanol; Sugar cane; Wheat

1. Introduction

Policosanol (PC) is the common name that refers to a mixture of long chain (20–36 carbon) aliphatic primary alcohols. The mixture contains mainly docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30).

Recently, literature on the role of PC in prevention and treatment of cardiovascular disease was reviewed (Varady, Wang, & Jones, 2003). An overview of the beneficial health effects of C28 has also been reported (Taylor, Rapport, & Lockwood, 2003). Policosanol has been shown to decrease platelet aggregation, endothelial damage, and foam cell formation (Arruzazabala, Carbajal, R., Garcia, & Fraga, 1993; Carbajal, Arruzazabala, Valdes, & Mas, 1998). The effectiveness of policosanol as a lipid-lowering agent, in several different populations has been extensively studied (Castano et al., 1996, 2001; Gouni-Berthold & Berthold, 2002; Mas et al., 1999; Menendez et al., 1994). Research studies indicate that PC lowers cholesterol levels by inhibiting cholesterol biosynthesis and enhancing LDL decatabolism (Menendez et al., 1994). Policosanol inhibits lipid peroxidation. Policosanol improves protection of lipoproteins against lipid peroxidation, both in the lipid and the protein moieties (Menendez, Fraga, Amor, Gonzales, & Mas, 1999).

The acute and chronic toxicity, carcinogenicity, and mutagenicity of policosanol have been studied. Policosanol, administered to rats at 50–500 mg/kg-day for 12 months, revealed no treatment-related toxicity (Aleman et al., 1994). Furthermore, in vivo and in vitro studies showed no genotoxic effects on somatic or germinal cells

* Corresponding author. Tel.: +1 405 744 7062; fax: +1 405 744 6313.
E-mail address: nurhan.dunford@okstate.edu (N.T. Dunford).

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(Rendon et al., 1992). When used in long term clinical studies, policosanol has been shown to be well tolerated and safe (Mas et al., 1999; Pons & Rodriguez, 1994).

Currently, a number of dietary supplements containing PC are commercially available in the US market. The majority of these products are prepared from beeswax or sugar cane extracts. Although, wheat germ oil (WGO) contains PC, to our knowledge there is no commercial product containing wheat PC. A comprehensive study on the distribution of PC in the wheat plant has not been reported to date. Such a study is crucial for the evaluation of wheat PC as an alternative to current commercial PC source.

Thus, the main objective of this study is to compare the PC contents and compositions of beeswax, sugar cane and wheat extracts. The PC contents and composition of three commercial dietary supplements were also analyzed as reference commercial products.

2. Materials and methods

2.1. Materials

Wheat milling products (germ, bran, shorts, flour) were obtained from ADM Milling Corp. (Enid, OK). Crude WGO was a donation from Vitamins, Inc. (Chicago, IL). WGO-solids were the precipitate formed at the bottom of the oil container during storage. Sugar cane (Cubanfoodmarket.com, Miami, FL) and dietary PC supplements were commercial products purchased from US suppliers. Three different brands of dietary supplements, A, B and C, were examined for their PC contents and compositions. According to the product labels, PC contents in the products A and C were obtained from sugar cane and rice bran, respectively. The source of PC in supplement B was not declared on the label. The brown beeswax was obtained from Honey Hill Farm (Edmond, OK). The yellow beeswax bar was purchased from Activa Products, Inc. (Marshall, TX). Wheat straw was obtained from Oklahoma State University Experiment Station (Stillwater, OK).

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*-(trimethylsilyl) trifluoroacetamide (MSTFA), from Pierce (Rockford, USA), was used as the derivatization reagent. All other chemicals used in this study were reagent grade unless otherwise stated.

2.2. Analytical procedures

2.2.1. Standard solutions

Stock solutions of PC were prepared in chloroform (HPLC grade, Burdick & 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.

2.2.2. Sample Preparation

Wheat straw, wheat bran and wheat germ samples were ground in a coffee grinder (Black and Decker CBG5, Miami Lakes, FL) at medium speed for 1 min before use. Sugar cane was hand-peeled. The ground sample was hydrolyzed by refluxing with 1.0 N NaOH in methanol for 30 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 three times using equal volumes of diethyl ether. The diethyl ether phases collected from three extractions were combined and washed with Millipore water until reaching neutrality. The ether extract was evaporated to dryness under nitrogen 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 chloroform and 250 μl 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.

2.2.3. GC–MS analysis

Trimethylsilyl derivatives of alcohols were analyzed by an HP 6890 Series GC system coupled with a 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, USA). A fused silica capillary Equity-5 column (30 m × 0.25 mm × 0.5 μm film thickness) from Supelco (Bellefonte, USA) was used for the analysis. Oven temperature was programmed from 150 to 320 °C, with 4 °C/min heating rate, and maintained at 320 °C for 15 min. Helium was used as carrier gas at a flow rate of 1.0 ml/min. The inlet temperature was 300 °C. GC–MS operating temperatures were as follows: MS transfer line 280 °C, ion source 230 °C and MS quadrupole 150 °C. The ionization energy was 70 eV. The scan range and rate were 100–600 amu and 2 scans/s, respectively. The samples (1–2 μl) were 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 an HP Chemstation (Enhanced Chemstation G1701DA Version D.00.00.38, Agilent Technologies, Palo Alto, CA). The PC compositions of the samples

were 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).

2.3. 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). Multiple comparisons of the various means were carried out by least significant difference (LSD) test at $p = 0.05$.

3. Results and discussion

3.1. General

Total ion chromatograms for a standard mixture of PC and a typical wheat sample are shown in Figs. 1 and 2, respectively. The elution of the compounds from the capillary column was molecular weight dependent. The chromatogram for the sample was quite complicated and displayed numerous peaks (Fig. 2). The mass spectral library search indicated that some of the phytosterols, tocopherols and fatty acids in the sample mixture had either similar or slightly different retention times on the column than that of the PC. Hence, ''Extracted Ion Chromatograms,'' a tool on the Chemstation software, was used for the identification and extraction of the desired PC peaks from the other peaks on the sample chromatogram. The target and qualifier ions used for the extraction of individual PC peaks are shown in Table 1. The mass fractionation pattern of individual PC contents indicates that the target ion is formed by splitting a $-CH_3$ group from the PC chain (Fig. 3, Table 1).

3.2. Wheat

PC compositions of wheat samples are given in Table 2. The solid fraction that precipitated at the bottom of the container containing WGO (Crude WGO-solids) had the highest amount of total PC. The PC content of the clear WGO (oil above the precipitate) was about 17 times smaller than that of the WGO-solids/precipitate. Policosanol is part of the wax fraction which precipitates out from the crude oil during cold storage. Wheat straw had a significantly higher total PC content than did the other wheat milling fractions (wheat bran, germ, shorts and flour). This result was expected since PC is present in fruit, leaves and surfaces of plants, and whole seeds (Tulloch & Hoffman, 1973). Policosanol is a part of the protective and waterproofing surface layer, which acts as an interface between plant tissue and the growth environment (Walton, 1990). The very low (<1 mg/kg) PC content of wheat flour can be explained by the association of PC with plant surface layers and other lipophilic plant components rather than inner starchy endosperm. Wheat bran contains a significantly higher ($p < 0.05$) amount of PC than the germ. This might be due to the higher oil content of wheat germ

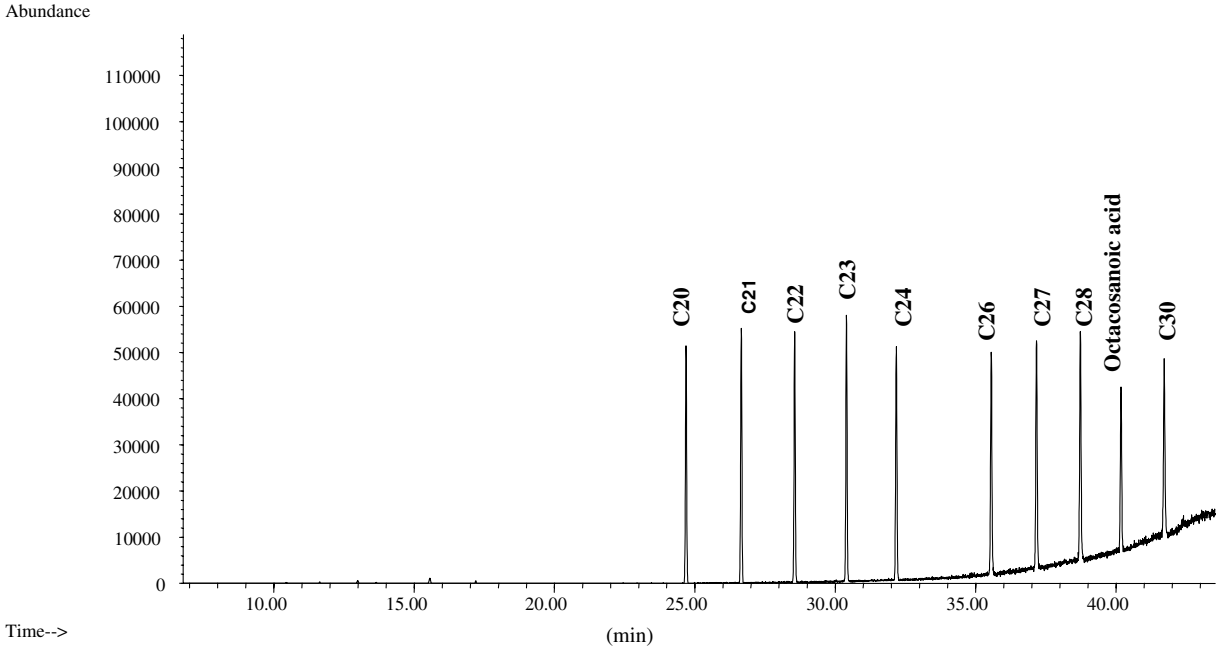

Fig. 1. Total ion chromatogram for a standard mixture of PCs.

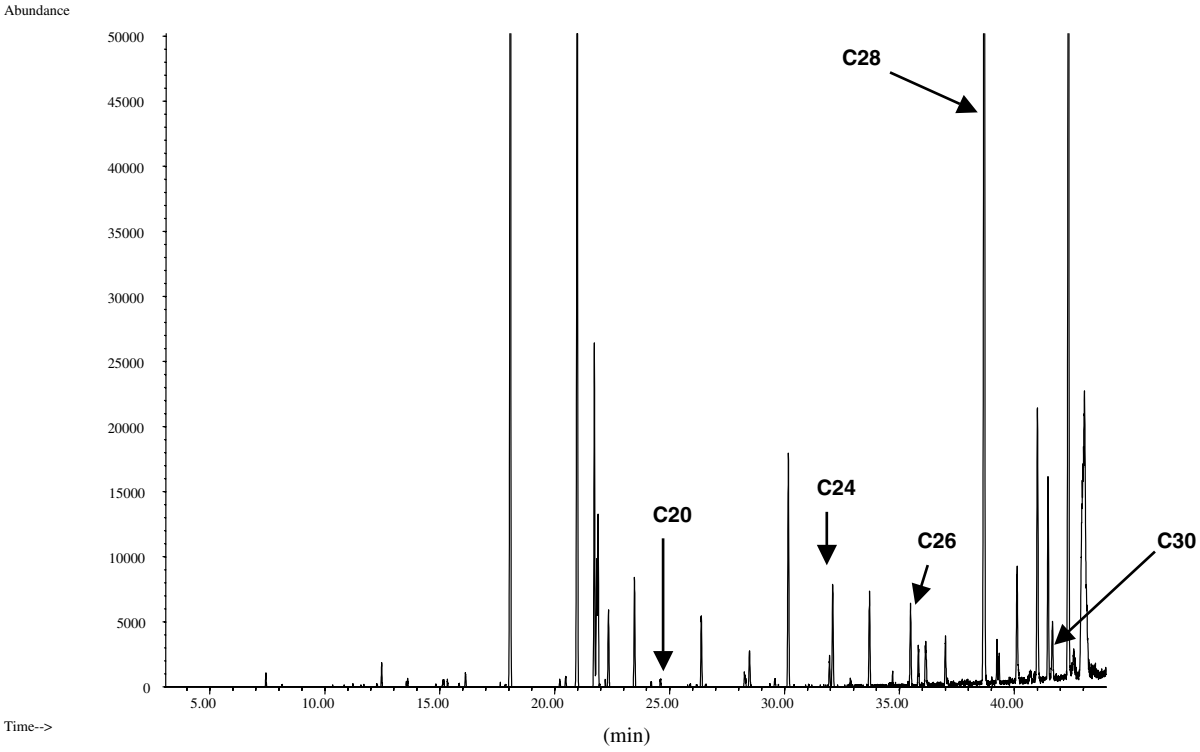

Fig. 2. A typical total ion chromatogram for a wheat sample.

Table 1
Mass fragmentation pattern of individual policosanols

	Target ion (*m*/*z*)	Qualifier ions (*m*/*z*)
Eicosanol (C20)	355.30	356.30; 103.0; 357.30
Heneicosanol (C21)	369.40	370.40; 103.0; 371.40
Docosanol (C22)	383.40	384.40; 103.0; 385.40
Tricosanol (C23)	397.40	398.40; 103.0; 399.40
Tetracosanol (C24)	411.40	412.40; 103.0; 413.40
Hexacosanol (C26)	439.50	440.50; 103.0; 441.50
Heptacosanol (C27)	453.50	454.50; 103.0; 455.50
Octacosanol (C28)	467.50	468.50; 103.0; 469.50
Triacontanol (C30)	495.50	496.50; 103.0; 497.50

(about 11%, w/w) than that of the bran (2–3%, w/w). Oil might have caused dilution of the PC in the germ extract.

PC compositions in wheat extracts and milling fractions varied significantly. The major PC in crude WGO-solids was C24 (34%, w/w, of the total PC). Hexa- and octa-cosanol are the two other PCs present in large quantities in the WGO-solids, 26 and 20% of the total PC, respectively. About 85% of the total PC in wheat straw was C28. The percentage of the other individual PC components in wheat straw was less than 10%.

3.3. Sugar cane

Sugar cane is the major source for the production of commercial PC-enriched products. Hence, a comparison of sugar cane PC content and composition with those of wheat is important for the evaluation of the potential of wheat as an alternative PC source. Policosanol content and compositions of whole raw sugar cane, peel from the sugar cane stem and sugar cane plant leaves were examined. The sugar cane peel contained the highest amount of total PC (about 270 mg/kg) (Table 3). The total PC content of sugar cane leaves (181 mg/kg) was quite similar to that of the wheat straw (164 mg/kg). Although, PC compositions of sugar cane plant parts varied significantly, C28 (about 81%) was the main component in all the sugar cane samples.

3.4. Beeswax

Some of the PC-containing dietary supplements are derived from beeswax. Total PC content of brown beeswax was about 20 and 45 times higher than those of the WGO-solids and sugar cane peel, respectively (Table 4). Yellow beeswax had significantly lower ($p < 0.05$) PC content than had brown beeswax. There were not enough data from the beeswax suppliers to explain the difference in PC content of the two samples. However, the lower PC content in the yellow beeswax might have been due to several parameters involved in hive formation and beeswax processing. Triacontanol (>40% of total PC) was the main component in both beeswax samples. A similar PC composition for beeswax was also reported in the literature (Jiménez, Bernal, Aumente, Toribio, & Bernal, 2003).

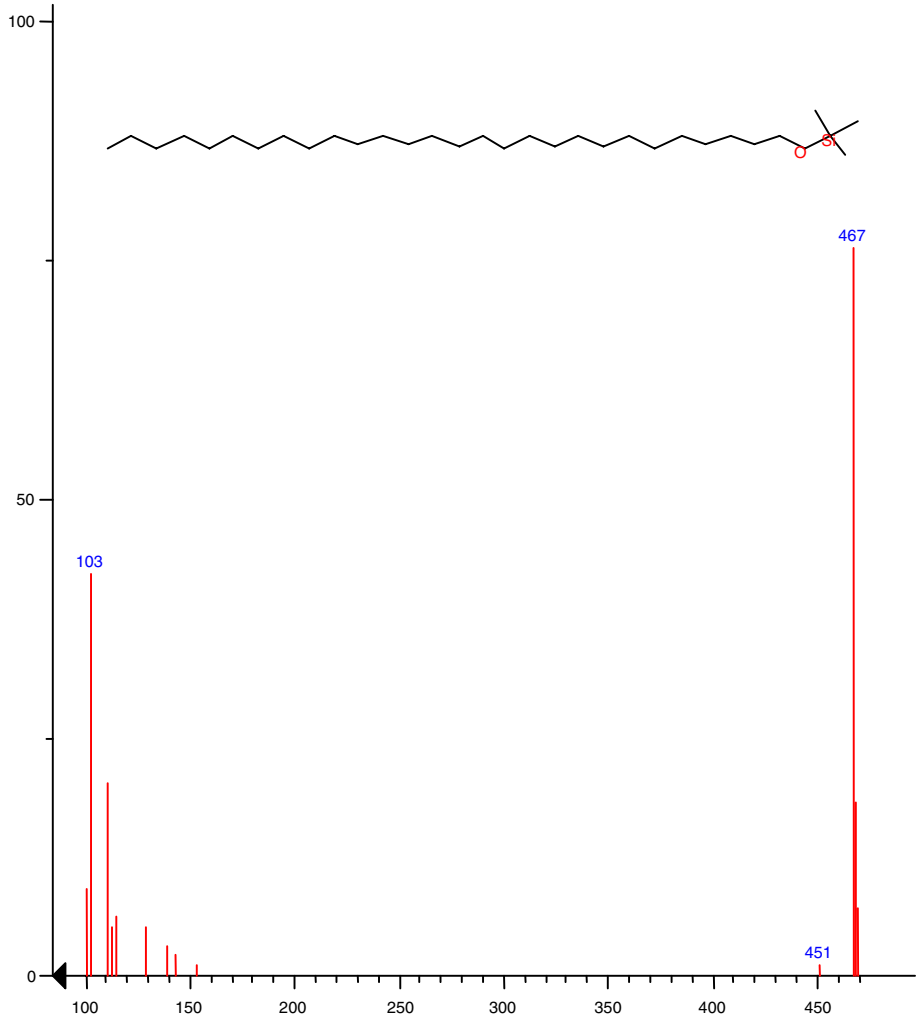

Fig. 3. Mass spectrum of octacosanol.

Table 2
Policosanol contents and composition of wheat fractions and wheat germ extracts[a]

Sample	Policosanol amount (mg/kg)					
	C22	C24	C26	C28	C30	Total PC[b]
Crude WGO-solids	47 ± 3	215 ± 10	164 ± 11	127 ± 8	55 ± 3	628 ± 17
Crude WGO-clear oil	2.8 ± 0.3	9.5 ± 0.9	8.1 ± 1.2	8.8 ± 0.4	5.8 ± 0.5	38 ± 2
Wheat straw	2.6 ± 0.3	10.7 ± 0.9	4.8 ± 0.6	140 ± 5	3.1 ± 0.7	164 ± 5
Wheat bran	2.73 ± 0.02	10.7 ± 0.01	4.87 ± 0.03	4.39 ± 0.02	n.d.[c]	30.0 ± 0.06
Wheat germ	2.8 ± 0.5	1.4 ± 0.2	n.d.[c]	2.9 ± 0.3	2.5 ± 0.3	10.0 ± 0.7
Wheat shorts	0.21 ± 0.02	0.82 ± 0.01	0.45 ± 0.03	0.39 ± 0.01	0.22 ± 0.03	3.29 ± 0.08
Wheat flour	n.d.[c]	n.d.[c]	n.d.[c]	0.17 ± 0.01	n.d.[c]	0.17 ± 0.01

[a] Docosanol (C22), Tetracosanol (C24), hexacosanol (C26), octacosanol (C28), Triacontanol (C30) and policosanol (PC).
[b] Total of 9 PC (C20, C21, C22, C23, C24, C26, C27, C28 and C30).
[c] Not detected.

3.5. Dietary supplements

Dietary supplements from three different suppliers were purchased over the internet and designated as A, B and C. The products B and C were in capsule and A was in tablet form. According to the product package labels, A contained 10 mg PC/tablet. However, our analysis results showed that each tablet contained only 5.3 mg PC (Table 5). About 58% of the total PC in product A was C28. The product B had the highest total PC content, 53.6 mg/capsule (Table 5). According to the package label, product B contained 20 mg C28/capsule,

Table 3
Policosanol contents and compositions of sugar cane[a]

Sample	Policosanol amount (mg/kg)					
	C22	C24	C26	C28	C30	Total PC[b]
Whole sugar cane	0.92 ± 0.07	1.68 ± 0.08	0.9 ± 0.2	10.0 ± 0.2	1.0 ± 0.2	17.4 ± 0.3
Sugar cane peel	2.4 ± 0.3	7.7 ± 0.2	23 ± 2	219 ± 3	16 ± 2	270 ± 4
Sugar cane leaves	9.9 ± 0.1	29.4 ± 0.5	22.4 ± 0.8	84 ± 4	26.6 ± 0.6	181 ± 4

[a] Docosanol (C22), Tetracosanol (C24), hexacosanol (C26), octacosanol (C28), Triacontanol (C30) and policosanol (PC).
[b] Total of 9 PC (C20, C21, C22, C23, C24, C26, C27, C28 and C30).

Table 4
Policosanol contents and compositions of beeswax from two different sources[a]

Sample	Policosanol amount (g/kg)					
	C22	C24	C26	C28	C30	Total PC[b]
Beeswax-brown	0.06 ± 0.01	2.6 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	5.7 ± 0.5	12.0 ± 0.6
Beeswax-yellow	n.d.[c]	1.11 ± 0.06	0.86 ± 0.07	0.90 ± 0.03	2.3 ± 0.4	5.2 ± 0.4

[a] Docosanol (C22), Tetracosanol (C24), hexacosanol (C26), octacosanol (C28), Triacontanol (C30) and policosanol (PC).
[b] Total of 9 PC (C20, C21, C22, C23, C24, C26, C27, C28 and C30).
[c] n.d. = Not detected.

Table 5
Policosanol content of commercial dietary supplements[a]

Sample	Policosanol (mg/tablet or capsule)						
	C20	C22	C24	C26	C28	C30	Total PC[b]
A (Tablet)	–	–	–	1.0 ± 0.2	3.1 ± 0.5	1.2 ± 0.2	5.3 ± 0.6
B (Capsule)	6.0 ± 0.2	7.1 ± 0.4	14.8 ± 0.5	8.5 ± 0.7	8.8 ± 0.6	8.3 ± 0.6	53.6 ± 1.3
C (Capsule)	–	–	0.58 ± 0.04	0.48 ± 0.02	0.94 ± 0.03	1.9 ± 0.1	3.9 ± 0.1

[a] Eicosanol (C20), Docosanol (C22), Tetracosanol (C24), hexacosanol (C26), octacosanol (C28), Triacontanol (C30) and policosanol (PC).
[b] Total of 9 PC (C20, C21, C22, C23, C24, C26, C27, C28 and C30).

but only 8.8 mg C28/capsule was detected. The C26, C28 and C30 contents in product B were quite similar, 15–16% of total PC. The major PC in product B was C24, 28% of total PC. The dietary supplement C package claimed 10 mg PC/capsule. However, our test results showed about 4 mg PC/capsule in this product and about 48% of the total PC in product C was C30 (Table 5).

4. Conclusions

Because of the complexity of the sample chromatograms, the analysis of individual PC components required mass spectrometric identification of the chromatographic peaks. Beeswax is the richest source of PC among the samples analyzed in this study. Wheat straw contained more PC than the wheat milling fractions. The total PC content of sugar cane peel and wheat straw were of the same order of magnitude. Policosanol compositions of the samples were source-dependent. The commercial dietary supplements contained less PC than was claimed on the labels. The PC compositions of the supplements varied significantly. These results emphasize the importance of regulation and monitoring of dietary supplements currently available in the market.

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