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Analytical Methods

Pressurised solvent extraction of policosanol from wheat straw, germ and bran

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ABSTRACT

Policosanols (PCs) are a group of long chain aliphatic alcohols that have been reported to have low-density lipoprotein (LDL) cholesterol-lowering properties. Wheat is a good source of these compounds. This study examined the effect of solvent type and temperature on extract yields and PC content and composition in the extracts. Wheat germ, straw and bran samples were extracted with petroleum ether, chloroform, *n*-hexane and ethanol at various temperatures ranging from 80 to 125 °C.

Wheat germ extract yields were higher than those for straw and bran. Ethanol extraction resulted in the highest yield from wheat germ. Ethanol extract yields from both wheat germ and straw increased significantly with increasing temperature. Wheat straw had the highest PC content among the wheat fractions examined in the study. The PC composition of extracts varied with the type of solvent and wheat fraction used. Ethanol and petroleum ether extracts of wheat straw had the highest octacosanol and hexacosanol contents, respectively. This study demonstrated that solvent type and temperature have significant effects on extract yields and PC composition in extracts obtained from wheat fractions.

1. Introduction

A number of animal and clinical studies have demonstrated that policosanol (PC) consumption may be effective in lowering low-density lipoprotein (LDL) and increasing high-density lipoprotein (HDL) cholesterol levels (Castano et al., 2001; Mas et al., 1999; Menendez, Fraga, Amor, Gonzalez, & Mas, 1999). There have also been reports indicating that cholesterol-lowering properties of PC are not reproducible in studies performed in the US (Jones, Kassis, & Marinangeli, 2009; Varady, Wang, & Jones, 2003). A better understanding of the effects of PC on disease prevention and treatment requires large scale independent animal and clinical studies involving various ethnic groups and subjects with different health histories. It is also critical that diets used in animal and clinical studies are well characterised for PC source and composition and presence of other bioactive compounds that may have synergistic effect. Furthermore type of PC delivery system such as capsules, tablets, emulsions and controlled release formulations may have significant effect on the efficacy and bioavailability of PC. Further research is needed to examine the effect of PC source, solvent type and extraction conditions on chemical composition of PC extracts and effect of these extracts on cardiovascular health.

PC containing dietary supplements in the form of capsules and tablets are available in health stores in the US. Currently sugar cane and beeswax are two main sources of commercial PC. Several studies reported the presence of PC in wheat leaf wax (Bianchi, 1985; Bianchi, Figrini, Borghi, & Corbellini, 1984; Tulloch & Hoffman, 1971, 1973). PC contents and compositions of wheat germ oil (WGO), straw and grain fractions have also been published (Irmak & Dunford, 2005; Irmak, Dunford, & Milligan, 2005). According to these studies wheat can be a good source of PC. The examination of wheat by-products such as straw, bran and germ as potential sources of PC will provide valuable information.

Pressurised liquid extraction (PLE), also referred to as accelerated solvent extraction (ASE), utilises liquid solvents at high temperature and pressure to achieve rapid extraction of compounds of interest from various materials. High temperature accelerates the extraction rate, while elevated pressure prevents boiling at temperatures above the normal boiling point of the solvent. It has been already shown that pressure does not have a significant effect on extraction other than keeping the solvent in the liquid phase (Vandenburg, Clifford, Bartle, & Shuang, 1998). When PLE/ASE is used time and solvent consumption are significantly reduced compared to the other solvent extraction techniques.

The selection of a suitable solvent is the most important step in optimising recovery of desirable components from a complex matrix. Solvent–solute interactions involve dispersion or London's forces and, very often, dipolar and/or multi-polar interactions. The dielectric constant is a measure of the polarity of a solvent and a key parameter in determining solute–solvent interactions. The dielectric constant of a solvent decreases with increasing

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temperature, consequently lowering the polarity of the solvent (Abboud & Notario, 1999). Thus temperature can be utilised to match the polarity of a solvent to that of the solute of interest to be recovered.

Automated laboratory scale ASE systems are helpful for screening biomaterials for their chemical composition, process parameters such as extraction temperature and solvent type to be used for isolation of the compounds of interest. The objective of this study was to examine the effect of solvent type and extraction temperature on PC recovery from wheat straw, germ and bran using pressurised solvent extraction.

2. Materials and methods

2.1. Materials

Wheat germ, bran and straw samples were examined as PC sources. Wheat germ was obtained from ADM Milling Corp. (Enid, OK) which mills a mixture of wheat varieties grown in Kansas and Oklahoma. The bran sample was from Trego wheat variety grown at Oklahoma State University, Stillwater Agronomy Research Station. The Trego wheat grain sample was milled at ADM Milling Corp. using a Buhler pilot mill (Buhler, Switzerland) to separate bran and endosperm fractions. Solvents used for oil extraction; *n*-hexane, ethanol, petroleum ether, chloroform (Burdick and Jackson, Muskegon, MI) were all reagent grade. The individual PC standards used for peak identification of eicosanol, heneicosanol, docosanol, tricosanol, tetracosanol, hexacosanol, heptacosanol, octacosanol were purchased from Sigma (Sigma–Aldrich Corporation, St. Louis, MO) and used without further purification (97% and higher purity). Triacontanol (96%) was obtained from Aldrich (Sigma–Aldrich Corporation, St. Louis, MO). N-Methyl-N-(trimethlysilyl)trifluoroacetamide from Pierce (Rockford, IL) was used as a silylation reagent. All other chemicals were reagent grade unless otherwise stated.

2.2. Accelerated solvent extraction

An accelerated solvent extraction unit, ASE 300 (Dionex Co., Sunnyvale, CA) was used for sample extraction. Details of the operation of the ASE system were reported elsewhere (Dunford & Zhang, 2003). The extractions of the samples were performed using four different solvents (*n*-hexane, ethanol, petroleum ether, chloroform) at five different temperatures (80, 90, 100, 110 and 125 °C) using 64 mL volume extraction cells. The extraction pressure, 1500 psi, was automatically maintained in the ASE 300 system. Each sample was extracted three times (three extraction cycles) for 15 min static time/cycle. Solvent was evaporated from the extract/solvent mixtures at 40 °C under vacuum using a Rapidvap evaporator (Labconco, Kansas City, MO) until constant weight was attained.

2.3. Analytical procedure

The extract was hydrolysed by refluxing with 1.0 M NaOH in methanol for 30 min. The mixture was cooled and Millipore water was added. Then, the solution was extracted with HPLC grade diethyl ether (Burdick and Jackson, Muskegon, MI). The extraction was performed three times using equal volumes of diethyl ether. The ether phase collected from three extractions was combined and washed with water until the pH of the water phase became neutral. Following drying over anhydrous sodium sulphate (ACS grade, EMD Chemicals Inc., Gibbstown, NJ) the ether extract was evaporated to dryness under nitrogen using a Reacti-Vap evaporation unit (Model 18780, Pierce, Rockford, IL). 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 20 min for derivatization. Chloroform was added to complete the volume to 1 mL before analysis.

PC compositions of the derivatized samples were analysed by a HP 6890 Series GC system coupled with 5973 network Mass Selective (MS) detector (Agilent Technologies, Palo Alto, CA). A fused silica capillary column, Equity-5 (30 m × 0.25 mm × 0.5 μm film thickness) from Supelco (Bellefonte, PA), was used. The GC oven temperature was programmed from 150 to 320 °C with 4 °C/min heating rate and maintained at this temperature for 15 min. Initial flow rate of the carrier gas, helium, was 1.0 mL/min. Inlet temperature was 300 °C. GC–MS parameters were as follows: MS transfer line temperature was 280 °C; ion source was kept at 230 °C and MS quadrupole temperature at 150 °C. The ionisation energy was 70 eV with 2 scans/s and mass range of 100–600 amu. The samples (1 or 2 μL) were injected into GC–MS with 1:10 split ratio. Data analysis was carried out by using HP Chemstation software. The PC compositions of the samples were identified by direct comparison of their chromatographic retention times and mass spectra with those of authentic compounds. The peaks were also confirmed with NIST/EPA/NIH Mass Spectral Library (Version 2.0).

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 comparison of the various means were carried out by LSD (Least Significant Difference) test at $\alpha = 0.05$.

4. Results and discussion

Solvents with a wide range of dielectric constants, hexane (1.9), petroleum ether (4.3), ethanol (24.3) and chloroform (4.8), were chosen for this study. The highest amount of extract was collected from wheat germ (Table 1). This was expected because wheat germ contains large amount of triacylglycerol (TAG), about 10–12% (w/w) (Dunford & Zhang, 2003). Low dielectric constant solvents used in this study (hexane, petroleum ether and chloroform) are capable of efficiently extracting lipids. The effect of solvent type on the amount of extract was significant only when wheat germ extracted with ethanol, 17.3% (w/w) which had the highest dielec-

Table 1
Effect of solvent type and temperature on the amount extract obtained from wheat germ, bran and straw.

Sample solvent/ temperature (°C)	Wheat germ (%, w/w)	Trego bran (%, w/w)	Wheat straw (%, w/w)
Petroleum ether			
80	10.3[a]	2.6[a]	1.7[a]
Chloroform			
80	10.1[a]	2.9[a]	1.9[a]
Hexane			
80	10.3[a]	2.5[a]	1.4[a]
Ethanol			
80	17.3[b]	3.0[a]	1.5[a]
90	17.7[b]	n.d.	4.2[b]
100	21[c]	n.d.	4.6[b]
110	n.d.	n.d.	5.1[c]
125	n.d.	n.d.	5.8[d]

[a–d]Means in the same column with the same letter are not significantly different at $\alpha > 0.05$.
n.d., not determined.

Table 2
Effect of solvent on policosanol[a] content and composition of wheat straw extracts obtained at 80 °C.

Solvent	Policosanol amount (mg/100 g extract)					
	C22	C24	C26	C28	C30	Total PC[b]
Petroleum ether	95 ± 3	383 ± 15	606 ± 27	289 ± 45	189 ± 16	4272 ± 57
Chloroform	67 ± 1	254 ± 12	404 ± 16	166 ± 56	101 ± 3	2566 ± 60
Hexane	96 ± 1	370 ± 24	573 ± 14	263 ± 86	166 ± 9	3945 ± 91
Ethanol	37 ± 2	125 ± 2	176 ± 10	621 ± 14	38 ± 6	1036 ± 18

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

Table 3
Effect of temperature on policosanol composition[a] of wheat straw ethanol extracts.

Temperature (°C)	Policosanol amount (mg/100 g extract)					
	C22	C24	C26	C28	C30	Total PC[b]
80	37 ± 2	125 ± 2	176 ± 10	621 ± 14	38 ± 6	1036 ± 18
90	32 ± 8	83 ± 6	104 ± 17	492 ± 11	63 ± 7	811 ± 11
100	31 ± 1	70 ± 3	99 ± 4	428 ± 49	60 ± 12	723 ± 51
110	26 ± 2	60 ± 3	86 ± 6	440 ± 12	55 ± 4	696 ± 14
125	19 ± 1	47 ± 1	63 ± 2	285 ± 23	35 ± 6	470 ± 24

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

Table 4
Effect of solvent type on policosanol composition[a] of wheat germ extracts obtained at 80 °C.

Solvent	Policosanol composition (mg/100 extract)					
	C22	C23	C24	C26	C28	Total PC[b]
Chloroform	1.7 ± 0.02	2.7 ± 0.03	6.2 ± 0.01	6.5 ± 0.002	8.0 ± 0.004	27.6 ± 0.04
Hexane	1.8 ± 0.01	1.5 ± 0.05	6.1 ± 0.01	6.5 ± 0.003	8.6 ± 0.2	26.9 ± 0.2
Petroleum ether	1.6 ± 0.04	1.5 ± 0.01	5.9 ± 0.03	6.2 ± 0.04	7.0 ± 0.1	24.4 ± 0.1
Ethanol	1.3 ± 0.02	n.d.	6.4 ± 0.03	1.9 ± 0.2	7.5 ± 0.2	19.3 ± 0.3

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

Table 5
Effect of temperature on the policosanol composition[a] of wheat germ ethanol extracts.

Temperature (°C)	Policosanol composition (mg/100 mg extract)					
	C22	C24	C26	C27	C28	Total PC[b]
80	1.3 ± 0.02	6.4 ± 0.03	1.9 ± 0.2	0.68 ± 0.02	7.5 ± 0.2	19.3 ± 0.3
90	1.3 ± 0.02	6.1 ± 0.002	1.8 ± 0.03	0.6 ± 0.01	6.7 ± 0.01	18.4 ± 0.05
100	1.1 ± 0.01	5.3 ± 0.02	1.5 ± 0.003	0.6 ± 0.03	6.0 ± 0.05	15.8 ± 0.07

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

Table 6
Effect of solvent type on phytosterol content and composition[a] of Trego wheat bran extracts obtained at 80 °C.

Solvent/PC	C22	C24	C26	C28	C30	Total PC[b] (mg/100 g extract)
Petroleum ether	4.9 ± 1.0	12.2 ± 2.9	6.0 ± 1.9	6.3 ± 2.3	3.3 ± 1.1	37.9 ± 4.4
Chloroform	5.5 ± 0.7	13.7 ± 2.1	6.8 ± 1.4	7.3 ± 2.4	3.8 ± 1.3	43.2 ± 3.7
Hexane	4.9 ± 0.7	12.1 ± 1.5	6.1 ± 0.2	9.2 ± 4.4	3.5 ± 0.4	41.0 ± 4.7
Ethanol	3.7 ± 0.09	9.1 ± 0.3	4.5 ± 0.5	5.0 ± 1.4	2.4 ± 0.2	28.9 ± 1.5

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

tric constant and resulted in highest extract yield. It is expected that alcohol soluble proteins and other relatively polar wheat germ components are extracted with ethanol along with lipids. Ethanol extraction can be an advantage when simultaneous protein and lipid recovery is desired. Ethanol extract yields from both wheat germ and straw further improved with increasing temperature (Table 1). Wheat germ is the richest natural source of α-tocopherol, vitamin E, which is a heat sensitive compound. The highest

temperature examined for wheat germ extraction was limited to 100 °C in an effort to minimise tocopherol degradation (Eisenmenger & Dunford, 2008).

Petroleum ether and ethanol gave the highest and lowest, PC concentrations, respectively, in the straw extracts (Table 2). Ethanol extraction resulted in about 6% less PC recovery as compared to petroleum ether extraction. The major PC components in all extracts consisted of docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28) and triacontanol (C30). However, solvent type had a significant effect on the final PC composition in the extracts. The most abundant PC was C26 when non-polar solvents, petroleum ether, chloroform and hexane were used for extraction. C28 was the most abundant PC in ethanol extracts. About 43% more PC was recovered from straw at 125 °C as compared to that at 80 °C. The observed decrease in total PC concentration in extracts collected at high temperature was due to dilution caused by extraction of higher amounts of non-PC components under these conditions (Table 3). Increasing temperature did not have a significant effect on PC composition in the extracts. About 60% of the total PC was C28 in all straw ethanol extracts collected at different temperatures.

PC content of wheat germ extracts was significantly lower than that for straw. These results support the previously reported data on PC content of wheat straw and germ (Irmak & Dunford, 2005; Irmak, Dunford, & Milligan, 2005). Similar to the results obtained with wheat straw, ethanol extraction of germ resulted in the lowest PC concentrations among the solvents examined in the current study (Table 4). Total PC recovery was highest when germ was extracted with ethanol. Triacontanol (C30) content of all germ extracts was below the detection limit of the analytical protocol used for PC analysis in this study. Hexacosanol (C26) content in wheat germ ethanol extracts was significantly lower than that in chloroform, petroleum ether and hexane extracts. Total PC concentration in ethanol extracts decreased significantly with increasing temperature. There was no significant difference in total PC recovery with temperature (Table 5). Lower PC concentration can be explained with dilution effect caused by the presence of larger amount of non-PC components in the extracts collected at high temperatures.

Total PC content of Trego wheat bran extracts was higher than that for germ but lower than straw extracts (Table 6). Ethanol extraction resulted in lowest PC concentration. C22, C24, C26, C28 and C30 were the major PC found in all extracts. C24 was the most abundant PC in all bran extracts.

This study demonstrated that type of solvent and extraction temperature had significant effects on extract yields and PC composition. There were significant differences in PC content and composition among wheat fractions, bran, germ and straw.

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