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ARTICLE

Effect of the Solvent Type and Temperature on Phytosterol Contents and Compositions of Wheat Straw, Bran, and Germ Extracts

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Wheat fractions, such as bran, germ, and straw, are rich in a number of health beneficial bioactive compounds. However, they have not been exploited to their full capacity for value-added product development. This study examines the potential of recovering phytosterol (PS)-enriched extracts from wheat germ, bran, and straw. The main objective of the study was to evaluate the effect of solvent type and temperature on PS content and composition in straw, bran, and germ extracts. Petroleum ether, chloroform, *n*-hexane, and ethanol were used as solvents. A pressurized solvent extraction system was used for extraction of wheat fractions. Germ extracts had the highest total PS content followed by straw and bran extracts. β-Sitosterol, campesterol, and stigmasterol were the main PSs in all of the extracts. Ethanol extraction resulted in the lowest total PS recovery from germ. Solvent type had a significant effect on PS composition in straw extracts. β-Sitosterol was the most abundant PS in straw hexane extracts (74% of total PS). Petroleum ether, chloroform, and ethanol extracted more stigmasterol than β-sitosterol from straw. This study demonstrated that the solvent type and temperature had significant effects on both PS content and composition of extracts collected from wheat fractions. Because of the complex nature of the agricultural materials, solvent selection and process optimization need to be based on experimental data. Pressurized solvent extraction is a useful technique to screen complex biological materials for their composition and to determine processing conditions to be optimized.

KEYWORDS: Phytosterols; wheat; germ; straw; bran; pressurized solvent extraction

INTRODUCTION

Plant sterols, also referred to as phytosterols (PSs), are essential constituents of plant cell membranes because of their membrane-stabilizing effect (*1*). PSs are well-known for their cholesterol-lowering properties (*2*−*4*). PSs have received U.S. Food and Drug Administration (FDA) clearance for generally recognized as safe (GRAS) status. In the U.S., foods containing plant sterols can carry health claims indicating their cholesterol-lowering properties (*5*).

Wheat is rich in a number of health beneficial bioactive compounds (*6*). Wheat germ, bran, and straw are byproducts from harvest and milling operations. The PS content and composition of wheat grain fractions and wheat germ oil (WGO) have been reported by several research groups (*7*−*15*). The PS content of whole wheat grain is lower than that of the bran and germ fractions (*15*). It is well-established that the germ and bran fractions of the wheat grain are rich in health beneficial compounds (*16*, *17*). Wheat germ oil contains a significantly higher amount of PS than the other common commercial oils (*7*). The presence of PS in wheat straw has also been reported (*18*, *19*).

In general, commercial PS-enriched food products are made with PS isolated from deodorizer distillate, which is a byproduct of vegetable oil refining. Extracts concentrated in PS can also be obtained from wheat bran, straw, and germ. Selection of a suitable solvent is the most important step in optimizing the recovery of desirable components from a complex matrix. Solvent−solute interactions always involve dispersion or London's forces and, very often, dipolar and/or multipolar interactions. The dielectric constant is a measure of the polarity of the solvent and a key parameter in determining solute−solvent interactions. The dielectric constant of a solvent decreases with increasing temperature, consequently lowering the polarity of the solvent (*20*). This property can be used to match the polarity of a solvent to that of the solute of interest to be recovered by adjusting the temperature of the system. The solubility of a solute in a solvent can also be estimated by the use of solubility parameters. In general, the closer the solubility parameters, the more solute will dissolve in a solvent (*21*). Although there are models to predict the solubility parameter, precise quantitative relationships between the solubility parameter and extraction efficiency from complex biological samples is not yet possible (*21*). Hence, the optimization of recovery of components of interest

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from agricultural materials is usually based on experimentation. Laboratory-scale pressurized solvent extraction (PSE) also referred to as accelerated solvent extraction (ASE) systems are helpful for screening suitable solvents and extraction conditions for a given application (*22*). PSE reduces both time and solvent required to recover compounds of interest. The objective of this study was to use PSE to examine the effect of solvent type and temperature on PS recovery from wheat germ, bran, and straw.

MATERIALS AND METHODS

Materials. Wheat germ was obtained from the ADM Milling Corp. (Enid, OK), which mills a mixture of wheat varieties grown in Kansas and Oklahoma. The straw and bran samples were from Trego wheat variety grown at the Stillwater Agronomy Research Station, Oklahoma State University. The Trego grain sample was milled at the ADM Milling Corp. using a Buhler pilot mill (Buhler, Switzerland) to separate bran and endosperm fractions. Reagent-grade ethanol, *n*-hexane, petroleum ether, and chloroform (Burdick and Jackson, Muskegon, MI) were used for oil extraction. The phytosterol standards were stigmasterol (95% purity), β-sitosterol (97% purity) (Sigma-Aldrich Corporation, St. Louis, MO), and campesterol (Matreya, Inc., Pleasant Gap, PA). Stock solutions of PS were prepared in chloroform (HPLC-grade, Burdick and Jackson, Muskegon, MI) and derivatized at 60 °C for 15−20 min. *N*-Methyl-*N*-(trimethlysilyl)trifluoroacetamide from Pierce (Rockford, IL) was used as a silylation reagent. The desired concentrations of standard solutions were prepared by dilution of the stock solutions. All other chemicals were reagent-grade unless otherwise stated.

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 (*22*). The extractions of the samples were performed using four different solvents (*n*-hexane, ethanol, petroleum ether, and chloroform) at five different temperatures (80, 90, 100, 110, and 125 °C) using about 20 g of sample in 64 mL volume extraction cells. The extraction pressure, 1500 psi, was automatically maintained in the ASE 300 system. Each sample was extracted 3 times (three extraction cycles) for 15 min of static time/cycle. The solvent was evaporated from the extract/solvent mixtures at 40 °C under vacuum using a Rapidvap evaporator (Labconco, Kansas City, MO) until a constant weight was attained.

Analytical Procedure. The extract 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 funnel. Residual material on the glass wool was washed with deionized water obtained from a Millipore water purification system (Millipore, Billerica, MA). Then, the solution was extracted with HPLC-grade diethyl ether (Burdick and Jackson, Muskegon, MI). The extraction was performed 3 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. After drying over anhydrous sodium sulfate (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 of chloroform and 250 μL of 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.

PS compositions of the derivatized samples were analyzed by a HP 6890 series GC system coupled with a 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 a 4 °C/min heating rate and maintained at the latter temperature for 15 min. The initial flow rate of the carrier gas, helium, was 1.0 mL/min. The 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 was kept at 150 °C. The ionization energy was 70 eV with 2 scans/s and a mass range of 100−600 amu. The samples (1 or 2 μL) were injected into GC−MS with a 1:10 split ratio. Data analysis was carried out using HP Chemstation software. The PS compositions of the samples were identified by a 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).

Table 1. Effect of Solvent Type on Phytosterol Content and Composition of Wheat Straw Extracts Collected at 80 °C

	phytosterol composition (mg/100 g of extract)			
solvent	β-sitosterol	campesterol	stigmasterol	total PS
petroleum ether	86 ± 2	27 ± 9	194 ± 6	307 ± 7
chloroform	78 ± 2	24 ± 9	180 ± 7	282 ± 8
hexane	191 ± 5	33 ± 1	35 ± 2	258 ± 5
ethanol	119 ± 10	26 ± 5	168 ± 2	313 ± 3

Table 2. Effect of the Temperature on the Phytosterol Composition of Wheat Straw Ethanol Extracts

	phytosterol amount (mg/100 g of extract)			
temperature (°C)	β-sitosterol	campesterol	stigmasterol	total PS
80	119 ± 10	26 ± 5	168 ± 2	313 ± 3
90	490 ± 93	85 ± 13	355 ± 62	930 ± 11
100	390 ± 19	134 ± 16	271 ± 11	795 ± 28
110	381 ± 19	130 ± 12	247 ± 17	758 ± 28
125	283 ± 7	92 ± 3	174 ± 8	549 ± 11

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 a least significant difference (LSD) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The effects of temperature and solvent type on wheat germ, bran, and straw extract yields were reported in an earlier study (*23*). The highest extract yield was obtained with wheat germ, followed by bran and straw. Increasing the extraction temperature resulted in higher extract amounts. In this study, the PS content and composition of the same extracts are reported.

The PS contents of wheat straw petroleum ether and ethanol extracts were significantly higher ($p < 0.05$) than those of chloroform and hexane extracts (**Table 1**). Stigmasterol, β-sitosterol, and campesterol were the main PSs detected in all of the extracts. Solvent type had a significant effect on PS composition in straw extracts. About 63% (w/w) of the total PS was stigmasterol when petroleum ether and chloroform were used as the solvent. In hexane extracts, about 74% of the total PS was β-sitosterol. Ethanol extracts consisted of about 54% stigmasterol, 38% β-sitosterol, and 8% campesterol (**Table 1**).

The total PS content of straw ethanol extracts significantly increased when the temperature was raised from 80 to 90 °C (**Table 2**). A further increase in temperature above 90 °C caused a significant decrease in the PS content in the extracts. The PS recovery from the samples at a given temperature and solvent was calculated as follows: (mg of extract/100 g of germ, bran, or straw) × (mg of PS in the extract/100 g of extract). Extract yields were 4.2 g/100 g of straw at 90 °C and 5.8 g/100 g of straw at 125 °C when ethanol was used as the solvent (*23*). The highest PS recovery with ethanol, 0.39 mg/g of straw, was achieved at 90 °C. This result indicates that the decrease in the PS concentration with an increasing temperature was due to the extraction of a higher amount of non-PS components at higher temperatures, leading to dilution of the PS concentration in the extracts.

Total PS contents of wheat germ extracts were significantly higher than those of straw extracts (**Table 3**). Ethanol extraction

Table 3. Effect of the Solvent Type on the Phytosterol Content and Composition of Wheat Germ Extracts Obtained at 80 °C

	phytosterol composition (mg/100 g of extract)			
solvent	β-sitosterol	campesterol	stigmasterol	total PS
petroleum ether	1523 ± 30	628 ± 24	21 ± 2	2172 ± 38
chloroform	1790 ± 20	706 ± 33	28 ± 1	2524 ± 39
hexane	1782 ± 21	724 ± 11	26 ± 1	2532 ± 24
ethanol	495 ± 1	185 ± 2	16 ± 0.4	696 ± 2

Table 4. Effect of the Temperature on the Phytosterol Content and Composition of Wheat Germ Ethanol Extracts

	phytosterol amount (mg/100 g of extract)			
temperature (°C)	β-sitosterol	campesterol	stigmasterol	total PS
80	495 ± 1	185 ± 2	16 ± 1	696 ± 2
90	604 ± 2	223 ± 2	16 ± 1	844 ± 3
100	536 ± 1	200 ± 3	15 ± 0.4	751 ± 3

Table 5. Effect of the Solvent Type on the Phytosterol Content and Composition of Trego Bran Extracts Obtained at 80 °C

	phytosterol composition (mg/100 g of extract)			
solvent	β-sitosterol	campesterol	stigmasterol	total PS
petroleum ether	70 ± 4	20 ± 6	21 ± 3	111 ± 8
chloroform	95 ± 5	22 ± 5	25 ± 3	141 ± 5
hexane	89 ± 8	18 ± 1	19 ± 3	127 ± 8
ethanol	103 ± 1	15 ± 2	17 ± 2	135 ± 2

resulted in the lowest total PS concentration in the extracts. In a previous study, we have shown that ethanol, chloroform, and hexane wheat germ extract yields were 17.3, 10.1, and 10.3 g/100 g of wheat germ, respectively (*23*). On the basis of this data, chloroform and hexane extraction resulted in about 46% higher total PS recovery than ethanol. β-Sitosterol was the main PS in all of the extracts. Solvent type did not have a significant effect ($p > 0.05$) on the PS concentration in the extracts (**Table 3**). The highest total PS concentration was achieved at 90 °C when ethanol was used as the solvent (**Table 4**). Although the total PS concentration in germ ethanol extracts obtained at 100 °C was lower than that collected at 90 °C, differences in total PS recovery attained at both temperatures were not statistically significant because of the higher amount of extract collected at higher temperature, 17.7 and 21 g of extract/100 g of germ at 90 and 100 °C, respectively (*23*). The temperature did not have a significant effect ($p > 0.05$) on PS compositions in wheat germ ethanol extracts (**Table 4**). About 71% of the total PS was β-sitosterol in all of the extracts.

Trego bran extracts had the lowest total PS concentrations among the wheat fractions examined in this study (**Table 5**). Chloroform extracted the most PS from Trego bran. Although β-sitosterol was the most abundant PS in all extracts, ethanol and petroleum ether extracts had the highest and lowest β-sitosterol concentration, 76 and 63% of the total PS in the extracts, respectively. Stigmasterol and campesterol concentrations of all of the extracts were similar.

To the best of our knowledge this is the first study that examines the effect of the solvent type and extraction temperature on PS recovery from wheat fractions. This study clearly demonstrated that wheat germ was a better PS source than straw and bran. This result is expected because wheat germ has a higher oil content than straw and bran and PSs are associated with oil. Nonpolar solvents, such as petroleum ether, chloroform, and hexane, were more effective than relatively polar solvent ethanol in PS recovery from wheat germ. Although PS contents of straw extracts were higher than those for bran, PS recoveries from both sources were similar because of higher extract yields obtained from bran than those from straw. The optimum temperature for PS recovery from both straw and germ was 90 °C when ethanol was used for extraction. Higher temperatures increased extract yields. However, PS recovery did not improve significantly ($p > 0.05$) with an increasing extraction temperature. The data presented in this study would be valuable for choosing feedstock and processing parameters for PS recovery from wheat fractions.

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