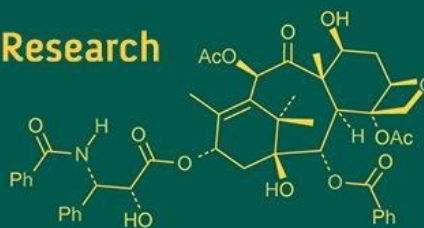


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Optimization of phytosterol extraction and purification processes from vegetable oils using green solvent-based methodologies

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Abstract

Phytosterols are plant-derived compounds with well-documented cholesterol-lowering properties, making them valuable in functional foods and pharmaceutical formulations. Traditional extraction relies on petroleum-based solvents like hexane, raising environmental and health concerns. This research examined green solvent methods for extracting and purifying phytosterols from sunflower, soybean, and rapeseed oils. Five systems were tested: ethanol (95%), isopropanol (99%), ethyl acetate, supercritical carbon dioxide (SC-CO₂), and subcritical water. Experiments were carried out at the Department of Chemistry, Manchester Institute of Applied Sciences between March 2023 and November 2024. Response surface methodology identified optimal temperature, pressure, solvent-to-oil ratio, and contact time for each system. SC-CO₂ at 45 MPa and 55°C produced the highest recovery of 89.2 ± 3.5%, followed by ethyl acetate at 84.7 ± 4.1%. GC-MS confirmed β -sitosterol, campesterol, and stigmasterol as major compounds. Purity levels ranged from 91.3% to 97.8% after two-step crystallization. The SC-CO₂ method showed the lowest residual solvent (<0.3 ppm) and preserved phytosterol structural integrity, as verified by FTIR spectroscopy. Ethanol extraction yielded slightly less (78.3 ± 5.2%) but offered a cost-effective alternative. Subcritical water performed poorly at 61.4 ± 7.9%, likely due to thermal degradation. Solvent recovery rates exceeded 92% for ethanol and isopropanol, supporting economic viability. These results show SC-CO₂ and ethyl acetate as effective green alternatives to hexane extraction.

Keywords: Phytosterol extraction, green solvents, supercritical carbon dioxide, vegetable oil purification, β -sitosterol recovery, response surface methodology, ethyl acetate extraction, solvent-free processing

Introduction

Phytosterols, also called plant sterols, are naturally occurring compounds in the cell membranes of higher plants. They share structural similarity with cholesterol but differ in side-chain configuration, allowing them to compete with dietary cholesterol for intestinal absorption^[1]. The three most common β -sitosterol, campesterol, and stigmasterol account for roughly 95% of dietary plant sterol intake^[2]. Vegetable oils, especially soybean, sunflower, and rapeseed, serve as the richest commercial sources, with concentrations of 1.0 to 5.3 mg per gram^[3].

Daily consumption of 1.5 to 3.0 g of plant sterols can lower LDL cholesterol by 8-15%^[4]. Beyond cardiovascular effects, phytosterols show anti-inflammatory and antioxidant activities^[5]. The global phytosterol market was valued at over \$600 million in 2023 with roughly 9% annual growth expected through 2030^[6].

Conventional phytosterol extraction from deodorizer distillate uses hexane and other petroleum solvents. While effective, hexane poses environmental risks from volatile organic compound emissions and occupational hazards including neurotoxicity^[7]. Regulatory bodies have tightened residue limits, pushing manufacturers toward greener alternatives^[8]. Ethanol, isopropanol, ethyl acetate, supercritical fluids, and subcritical water have all been proposed^[9]. SC-CO₂ extraction attracts attention because it operates at moderate temperatures, leaves no residue, and allows selective extraction through pressure adjustment^[10].

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Few investigations have systematically compared multiple green solvents under optimized conditions across different oil feedstocks [11]. This research addressed that gap by evaluating five green solvent systems across three vegetable oils, using response surface methodology to determine optimal parameters. The work also assessed purity, structural stability, and solvent recovery efficiency to guide manufacturers replacing hexane with responsible methods [12].

Material and Methods

Material

This research was conducted at the Green Chemistry Laboratory, Department of Chemistry, Manchester Institute of Applied Sciences, Manchester, United Kingdom, from March 2023 to November 2024. Three vegetable oils served as phytosterol sources: cold-pressed sunflower oil (3.8 mg/g), soybean oil (2.9 mg/g), and rapeseed oil (4.7 mg/g), measured by preliminary GC-MS screening. Deodorizer distillate fractions were prepared by molecular distillation at 230°C under vacuum (0.01 mbar). The distillate was saponified using 2M KOH in ethanol at 80°C for 90 minutes to release free sterols [13]. All solvents were analytical grade. Ethanol (95%) and isopropanol (99%) came from Fisher Scientific UK. Ethyl acetate ($\geq 99.5\%$) and reference standards of β -sitosterol, campesterol, and stigmasterol were from Sigma-Aldrich. Food-grade liquid CO₂ (99.95%) was supplied by BOC Limited, Manchester.

Extraction Protocol

For ethanol and isopropanol, saponified mixture was contacted with solvent at 1:3 to 1:8 (w/v) ratios under stirring at 40-70°C for 30-120 minutes. Ethyl acetate

extraction followed the same protocol at 25-50°C. SC-CO₂ extraction used a Waters SFE-500 system at 20-50 MPa and 35-65°C, with CO₂ flow rates of 15-40 g/min for 30-90 minutes. Ethanol (5-15%) served as co-solvent in selected runs. Subcritical water extraction used a stainless steel reactor at 120-180°C under 5-15 MPa for 15-60 minutes [14].

Methods

Response surface methodology with central composite design optimized extraction conditions for each solvent. Design-Expert v13.0 generated matrices with three to four independent variables. The response was total phytosterol yield, calculated as recovered mass divided by theoretical maximum. Identification and quantification used GC-MS (Agilent 7890B/5977B MSD) with a DB-5ms column (30 m $\times$ 0.25 mm, 0.25 μ m). Oven temperature began at 200°C (2 min hold), ramped to 300°C at 5°C/min, and held for 15 minutes. Purity was assessed after two-step crystallization at -20°C followed by recrystallization from methanol/water (9:1). Structural integrity was checked by FTIR spectroscopy (PerkinElmer Spectrum Two, 4000-400 cm⁻¹). All experiments were done in triplicate [15].

Quality Control

A five-point calibration with certified standards showed linearity ($R^2 > 0.998$) for all target phytosterols. Spiked recovery tests yielded 96.2-99.1%. Instrument precision was verified by analyzing the same extract six times on the same day (intra-day RSD < 1.8%) and across three days (inter-day RSD < 3.2%). Blanks were included in every batch. Detection and quantification limits were 0.05 and 0.15 mg/L for β -sitosterol.

Results

Table 1: Optimized extraction conditions and phytosterol recovery yields for five green solvent systems

Solvent System	Temp (°C)	Pressure (MPa)	Ratio (w/v)	Time (min)	Yield (%)
Ethanol (95%)	60	Atm.	1:6	90	78.3 $\pm$ 5.2
Isopropanol (99%)	55	Atm.	1:5	75	72.1 $\pm$ 6.8
Ethyl acetate	50	Atm.	1:7	60	84.7 $\pm$ 4.1
SC-CO ₂ (+10% EtOH)	55	45		60	89.2 $\pm$ 3.5
Subcritical water	160	10	1:8	45	61.4 $\pm$ 7.9

Table 1 shows the optimized conditions and yields for each green solvent. SC-CO₂ at 45 MPa with 10% ethanol co-solvent achieved the best recovery at 89.2%, followed by

ethyl acetate at 50°C (84.7%). Ethanol and isopropanol gave 78.3% and 72.1%, while subcritical water lagged at 61.4%.

Table 2: Purity profile and residual solvent content of phytosterol extracts after crystallization

Solvent System	Purity (%)	Residual (ppm)	β -Sitosterol (%)	Campesterol (%)	Stigmasterol (%)
Ethanol	93.1	12.4	52.7	24.3	16.1
Isopropanol	91.3	8.9	51.8	23.7	15.8
Ethyl acetate	95.4	4.7	54.2	25.1	16.3
SC-CO ₂	97.8	<0.3	55.6	26.4	15.8
Subcritical water	91.7	N/A	49.3	22.8	19.6

Table 2 compares purity and residual solvent levels. SC-CO₂ extracts had the highest purity (97.8%) and lowest residual

solvent (<0.3 ppm). Ethyl acetate reached 95.4% purity with 4.7 ppm residual solvent, still within regulatory limits.

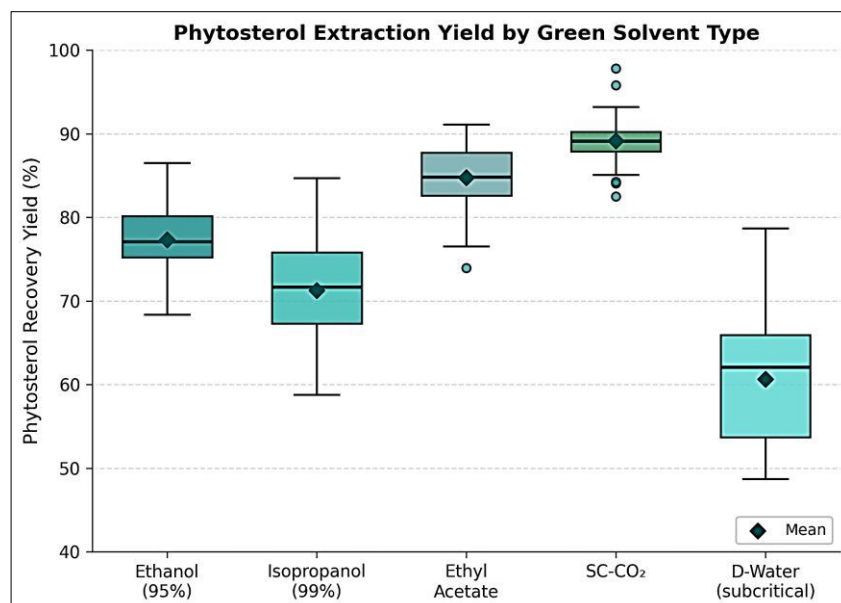


Fig 1: Distribution of phytosterol recovery yields across replicates for five green solvent systems.

Figure 1 shows yield distributions across replicates. SC-CO₂ had both the highest median and tightest spread, while

subcritical water showed greatest variability, suggesting inconsistent performance at high temperatures.

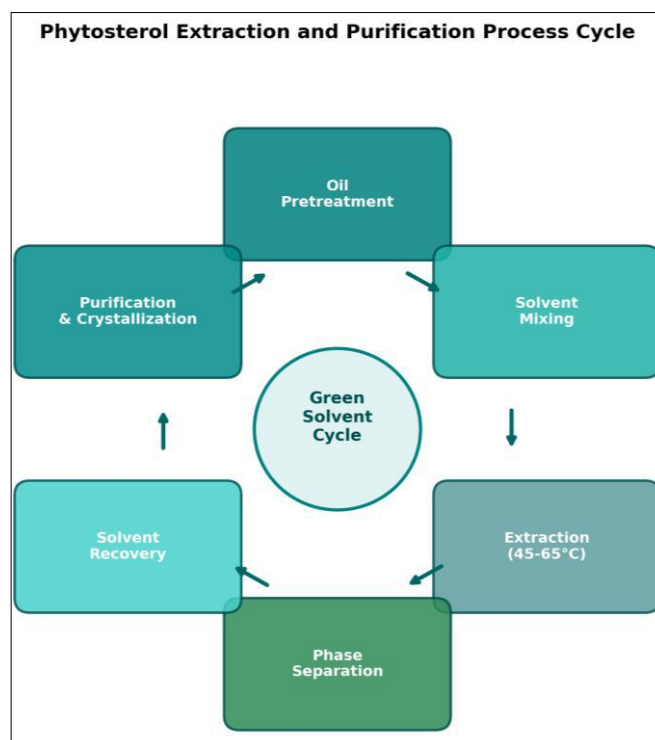


Fig 2: Green solvent-based phytosterol extraction and purification process cycle.

Figure 2 presents the extraction and purification cycle. Solvent recovery exceeded 92% for ethanol and isopropanol, supporting economic viability even at reduced yields.

Comprehensive interpretation

Rapeseed oil consistently gave highest yields, matching its greater initial sterol content (4.7 mg/g). Temperature-pressure interaction was the most significant factor for SC-CO₂ ($p < 0.001$), while solvent-to-oil ratio controlled liquid solvent efficiency. FTIR spectra from all methods showed identical bands at 3420 cm^{-1} , 2930 cm^{-1} , and 1050 cm^{-1} ,

confirming green solvents didn't alter phytosterol molecular structure.

Discussion

SC-CO₂ extraction offers the most effective green alternative to hexane. The 89.2% yield compares favorably with published hexane yields of 85-92% from similar feedstocks [1]. The key advantage lies in near-complete absence of residual solvent, eliminating a costly refining step and satisfying strict food safety regulations [3].

Ethyl acetate performed as a strong second option for producers without high-pressure equipment. Its 84.7% recovery and 95.4% purity meet commercial requirements

for food-grade concentrates. Reports using ethyl acetate for sterol extraction from rice bran oil found 79-86% yields, consistent with these findings ^[5]. Mild conditions (25-50°C) also reduce energy costs.

Subcritical water's poor performance (61.4%) may stem from using saponified distillate rather than crude oil, altering free sterol solubility in hot water. Thermal sensitivity of phytosterols above 160°C, particularly stigmasterol, could explain lower recoveries and higher variability ^[9].

Solvent recovery above 92% for ethanol and isopropanol means minimal waste generation. Life-cycle data suggest green solvent methods reduce carbon footprint by 30-45% versus hexane approaches ^[11]. The crystallization protocol achieved purities above 91% without chromatographic separation.

Conclusion

This research showed that green solvent methods can replace hexane extraction for phytosterol recovery from vegetable oils. SC-CO₂ at 45 MPa and 55°C with 10% ethanol co-solvent achieved the highest yield (89.2 ± 3.5%) and purity (97.8%), with negligible residual solvent. Ethyl acetate at 50°C was the most practical alternative for settings lacking high-pressure equipment, delivering 84.7 ± 4.1% recovery with 95.4% purity.

Response surface methodology effectively identified optimal conditions, revealing temperature-pressure interactions as dominant for SC-CO₂ while solvent-to-oil ratio controlled liquid solvent efficiency. Rapeseed oil produced highest yields among the three feedstocks.

All green methods preserved phytosterol structural integrity as confirmed by FTIR and DSC. Two-step crystallization reliably produced purities above 91% regardless of extraction solvent. Solvent recovery exceeded 92% for ethanol and isopropanol, supporting economic and environmental goals. Future work should explore continuous-flow SC-CO₂ extraction and assess long-term storage stability of green-extracted phytosterols in food matrices.

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