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# Application of green chemistry principles for extraction of phytolipids and phenolic compounds

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A new era of functional foods has resulted in increased interest in the omega-3 fatty acids, phenolic compounds, and other phytochemicals for preventing and controlling the development of cancer, cardiovascular and other diseases. The focus of the present research is to evaluate the influence of modern sample preparation technologies for extracting crude fat and phytochemicals from plant matrices utilizing fundamental principles of “green” chemistry. Optimization of extraction procedures using modern technologies significantly reduces the quantity of solvent consumed and waste generated during an extraction process. In addition, automation with modern instrumentation reduces exposure to extraction solvents and their vapour. This paper describes extraction of two classes of phytochemicals of extreme polarities, phytolipids (hydrophobic) and phenolics (hydrophilic). It compares five extraction technologies (Butt-tube, soxtec, pressurized liquid extractor, supercritical fluid extractor and Ankom batch extractor) for extraction of crude fat from three soy samples. In addition, this paper outlines a systematic approach for optimization of an extraction procedure for isolation of phytochemicals from different plant matrices using potentially bioactive phenolic compounds as a model. Applications of modern extraction technologies, in conjunction with optimized extraction procedures, will enable chemists and chemical engineers to considerably reduce the quantity of solvents consumed and waste generated during the extraction of bioactive phytochemicals from different plant matrices.

**Keywords**: Functional foods, omega-3 fatty acids, phytochemicals, Butt tube, Ankom batch extractor, green chemistry
**IPC Code**: Int.Cl.$^8$ A61K

It is well documented in archeological records that plants and plant products have been used for centuries for medicinal or health beneficial purposes[1]. Written records about medicinal plants date back at least 5000 years to the Sumerians[2]. This strong association between phytochemicals derived from plants and their health significance was somewhat weakened in the twentieth century after the introduction of synthetic aspirin in 1897 (ref. 2). Significant advancements and developments in the synthetic chemistry and pharmaceutical industries have been made since then. The discovery and benefits of modern drugs has primarily impacted the developed Western world. However, many developing and under-developed countries in the East still rely on ethnobotanical remedies for treatment of many illnesses[2].

Functional foods are often defined as foods or food components that provide health benefits beyond basic nutrition. With the introduction of the term functional foods in Japan during the mid-1980s and the remarkable advancements in the area of biotechnology, there has been renewed interest in the beneficial health properties exhibited by phytochemicals[3,4]. Several functional foods, such as calcium-fortified orange juice for maintaining bone health, omega-3 fatty acids for protecting against certain forms of cancer and cardiovascular diseases, and margarines formulated with cholesterol-lowering stanols, have been successfully introduced and are widely consumed by large populations around the world[5-8]. Even oatmeal (β-glucan, a soluble fiber)[8], cranberry juice (proanthocyanidins or condensed tannins)[9], soybean (isoflavones)[10], broccoli (selenium, phenolics, vitamins, dietary fiber and glucosinolates)[11,12], green tea (catechin and its analogs, polyphenolics)[13], and flaxseed (α-linolenic acid)[14] might be considered as functional foods as they are known to contain dietary components that can reduce risk of certain diseases. There has been a significant increase in demand for functional foods in the West during the past two decades due to increased health care costs, increased scientific evidence that diet can alter disease risk and progression, increased consumer interest, awareness and desire to enhance personal health, and changes in food regulations[15].

The demand for functional foods has generated interest in research on natural products, as plants are known to provide material with new structural features and novel bioactivities. Discovery of new natural products has also been possible due to rapid advancements in separation and analysis procedures. However, there is limited progress in improving the extraction process. Classical extraction procedures such as soxhlet, shaker, maceration, and percolation are still frequently used. These classical approaches used are manual processes that often require large volumes of organic solvents[16-18].

This in turn results in generation of large quantities of solvent waste that can cause significant environmental and health problems. This report describes extraction of two classes of phytochemicals of extreme polarities, phytolipids (hydrophobic) and phenolics (hydrophilic). It compares sample throughput, automation capabilities and quantity of solvent consumed by classical (Butt-tube) and modern [soxtec, pressurized liquid extractor (PLE), supercritical fluid extractor (SFE) and Ankom batch extractor (ABE)] as extraction technologies for the determination of phytofat from three soy samples. In addition, this paper outlines a systematic approach for optimization of an extraction procedure for isolation of potentially bioactive phenolic phytochemicals from different plant matrices using Black cohosh and parsley as examples. Optimization of an extraction procedure applying modern technologies allows researchers to reduce the usage and handling of extraction solvents and the waste generated during an extraction process.

## Materials and Methods

**Samples**. Three soy samples were obtained from Monsanto soy breeders (Ankeny, Iowa, USA). Fresh freeze-dried powder of Black cohosh from root and rhizome was obtained from David Lytle of the Eclectic Institute, Sandy, Oregon, USA. Dried parsley flakes were purchased from a local grocery store (Beltsville, Maryland, USA).

**Crude fat extraction from soy samples**. To eliminate the impact of particle size variations, all soybean seeds were ground on a Mega-grinder (Monsanto, St. Louis, USA). Ground soybean samples were extracted by five different extraction procedures (Butt-tube, soxtec , PLE, SFE, and ABE). The amount of crude fat extracted was determined gravimetrically after evaporation of the extraction solvent. Six replicate analyses were carried out for each sample by all five extraction procedures.

**Optimization of extraction procedures for extraction of phenolic phytochemicals from parsley flakes and black cohosh**. Optimization of different extraction parameters (solvent, extraction cycles, particle size, temperature, pressure, static time, solid-to-solvent ratio) for extraction of bioactive phenolic phytochemicals from two different plant matrices was carried out by using a pressurized liquid extractor. At least three replicate extractions were performed with each matrix with each variation of operating parameters. All extracts were assayed for total phenolic content by the Folin-Ciocalteu method and/or by HPLC with diode array detection. The identity of the phenolic compounds was determined by the LC-MS analysis or by comparison of their retention time and the UV-Vis spectra with authentic standards.

## Results and Discussion

**Table I** provides the comparison of sample throughput and automation capabilities of the five commonly used extraction procedures (Butt-tube, soxtec, PLE, SFE and ABE). The Butt-tube is a classical extraction procedure that is approved by American Oil Chemists Society for determination of crude fat (AOCS Ac 3-44)[16]. This method involves the continuous flow of a condensed extraction solvent over a ground sample matrix. Determination of crude fat for a 2-5 g sample size with the Butt-tube method requires approximately 225 mL organic solvent (petroleum ether or hexane) and the extraction time of 4-5 hr. This method can be replaced by newer techniques such as soxtec (a modified form of soxhlet procedure), PLE, SFE, or ABE. Application of modern techniques results in a significant reduction in the usage and handling of organic solvent and waste generated during an extraction process[17-22]. In addition, automation of the extraction process by

**Table I** — Comparison of sample throughput and automation capabilities of classical (Butt-tube) and modern (soxtec, accelerated solvent extractor (ASE), supercritical fluid extractor (SFE), and Ankom batch extractor (ABE)) extraction technologies

| Technology | Sample Throughput | Automation |
|---|---|---|
| Butt-tube | depends on the setup | no |
| Soxtec | 6 samples/1.25 hr | partial |
| ASE 200 | 24 samples/12 hr | yes |
| SFE-3560 | 24 samples/18 hr | yes |
| ABE | 20 samples/4 hr | yes |

newer techniques also reduces operator exposure to extraction solvents[20,21].

Soxtec is a modified soxhlet extraction method that was developed in the early 1970s by Edward Randall. In Randall's method, the sample is initially immersed in the hot boiling solvent followed by a rinsing step that flushes residual extract from the sample[20]. The amount of solvent consumed per extraction for a 2 g sample varies between 70-90 mL per sample as compared to 225 mL with a Butt-tube. Immersion in hot boiling solvent reduces the amount of solvent used per extraction and also decreases the extraction time. The newer model soxtec instrument has the capability of partially recycling the extraction solvents, thereby reducing the amount of solvent consumed per extraction.

Application of SFE or PLE also significantly reduces the extraction time, solvent consumed and the waste generated during an extraction process by extracting the sample at high temperature and pressure[19,21]. The solvent consumed per sample (1-2 g) for these methods generally varies between 0 to 25 mL per sample. Both SFE and PLE instruments have capabilities for extracting multiple samples in series or in parallel. Automation of the extraction and filtration processes with both PLE and SFE significantly reduces exposure to extraction solvent.

The ABE procedure allows parallel extraction of multiple samples (20) per batch[22]. Though a large volume of solvent (~ 1.5 L) is used for extraction per batch, over 90% of the solvent used during extraction can be recycled. The major disadvantage of ABE is that it is an indirect method, as percent crude fat is determined by the difference in weight of the fiber bag before and after the extraction. The oil extracted from individual samples cannot be further analyzed for its fatty acid methyl ester composition as the crude fat from multiple samples is pooled in one extract. The ABE is a beneficial technique for defatting samples, as defatted samples are contained in fiber bags that can be easily recovered for further analysis.

**Figure 1** shows the direct comparison of the percent crude fat extracted from three soy samples by the five different extraction techniques just described[18]. The yields of the total percent crude fat extracted by all five methods were quite similar (21.4% to 22.3%) with standard deviation of 0.1 to 0.2% (Ref. 18). However, in another study, it was observed that the yield of chlorogenic acid extracted from an eggplant sample varied significantly with the extraction method and conditions[23]. The maximum yield of chlorogenic acid was obtained when eggplant sample was extracted by the PLE procedure. Comparable yields (95-96%) of chlorgenic acid were obtained with both sonication and stirring extraction procedures. The yield of chlorogenic acid was reduced by 25%, as compared to PLE, when extractions were carried out under reflux with 85% aqueous methanol. This may be due to degradation of the chlorogenic acid at elevated temperatures. The lowest yield of chlorogenic acid was obtained when extraction was carried out on a rotary shaker with 50% aqueous methanol. This lower extraction yield of chlorogenic acid may be attributed to the combined effect of extraction solvent and conditions.

In recent publications, a systematic approach has been outlined for optimization of extraction procedures using phenolic compounds as a model phytochemical[24,25]. The first step is to select an appropriate solvent or solvent mixture that can extract the analyte of interest. Matching the polarity of the analyte of interest and extraction solvent is critical for optimum extraction. Selection of a 'green solvent' or solvent mixtures is preferred over chlorinated and aromatic organic solvents. The next step is to optimize extraction conditions (solvent mixture ratios, temperature, extraction cycles, matrix particle size and solid-to-solvent ratio, flush volume, pressure, and static time) which are method dependent. Optimization of extraction parameters not only increases extraction efficiency of the analyte of interest but also reduces the solvent consumed and the waste generated during an extraction process. Application of statistical experimental designs may further reduce the amount of solvent used as it minimizes the number of experiments required for optimization[26].

Solvent composition, temperature and number of extraction cycles must be effectively optimized to reduce the volume of solvent consumed and waste generated during an extraction process[25]. The effect of temperature on extraction efficiency is important as temperature impacts both analyte solubility and diffusibility through the porous sample matrix into the extraction solvent. In a previously reported study with black cohosh, an approximately 30% increase in extraction efficiency of total phenolics was observed when the temperature was increased from 40°C to 90°C[24]. A further 20°C increase in temperature to 110°C resulted in a 20% decrease that was attributed to

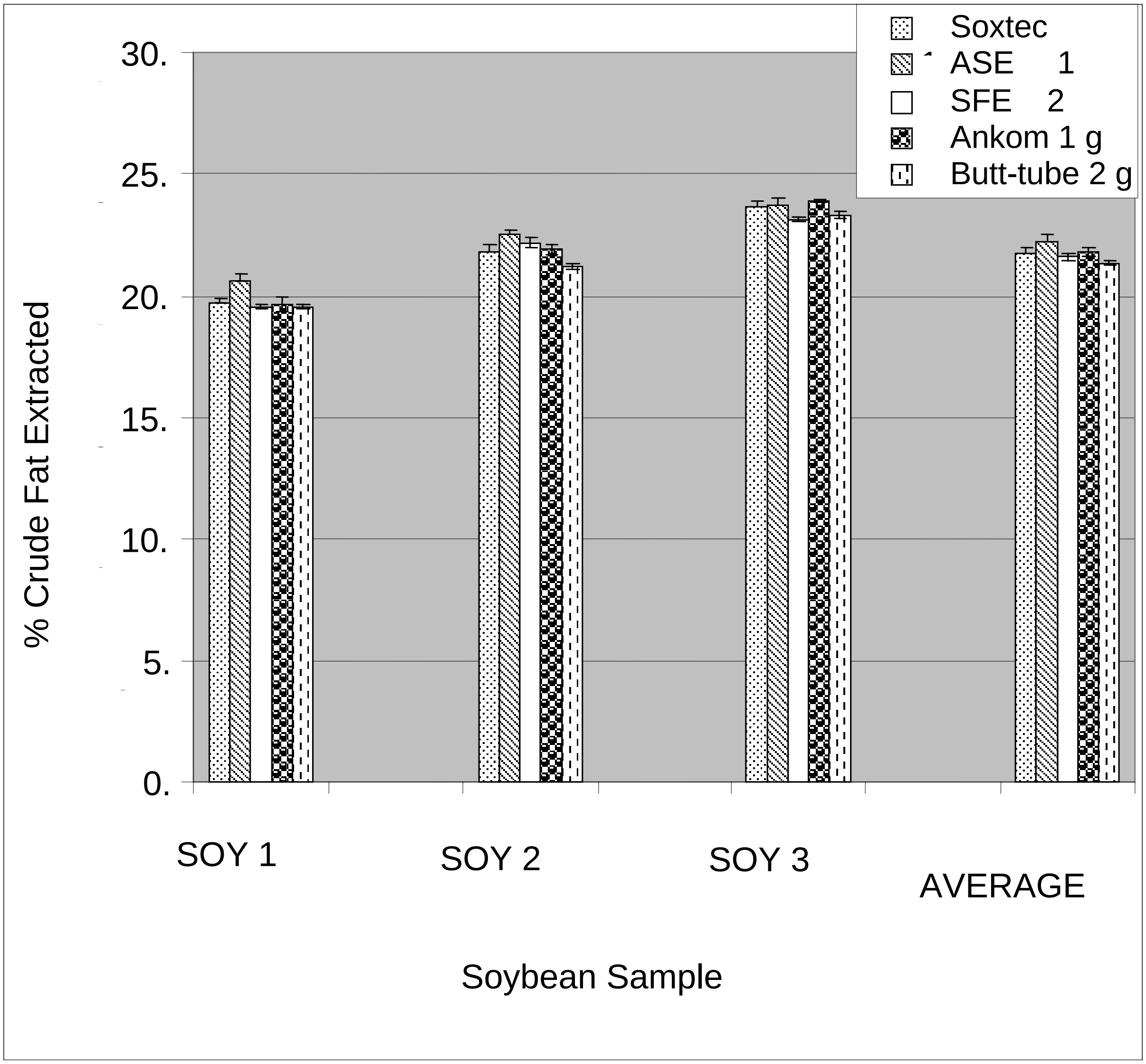

**Figure 1** — Comparison of the percent crude fat extracted from three soybean samples by classical (Butt-tube) and modern (soxtec, accelerated solvent extractor (ASE), supercritical fluid extractor (SFE), and Ankom batch extractor (ABE)) extraction procedures

the unstable nature of phenolics. The effect of the number of extraction cycles on the amount of phenolics isolated from parsley flakes is described in **Figure 2** (Ref. 25). In the previously reported study, over 88% of the phenolics were extracted in one cycle with acetone:$H_2O$ (5:5, v/v). However, only 42% of the total phenolics were extracted in the first cycle when parsley flakes were extracted with acetone:$H_2O$ (9:1, v/v).

Two other parameters that influence the quantity of solvent used during an extraction are the sample particle size and solid-to-solvent ratio. The influence of both these parameters on extraction efficiency of phytochemicals is often not well documented in the published literature. An approximate three-fold increase in extraction efficiency of total phenolics from black cohosh was observed when the particle size of the plant matrix was reduced from >2.0 mm to < 0.25 mm[23]. A decrease in particle size increases surface area and allows increased interaction between the sample matrix and the extraction solvent, which influences the extractability of the analyte of interest. Large variations in the solid-to-solvent ratio have been reported for extraction of phytochemicals[23]. Optimization of particle size and solid-to-solvent ratio not only increases extraction efficiency, but also reduces the quantity of solvent consumed and waste generated during extraction.

Three important parameters that are specific to the

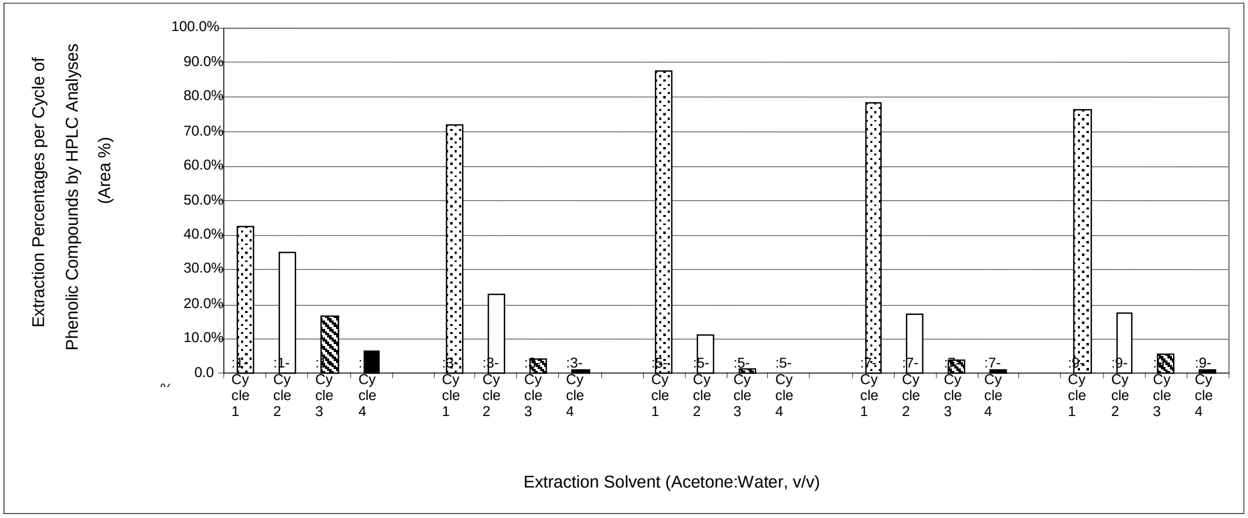

**Figure 2** — Influence of number of cycles on extraction percentages of phenolic compounds from ground parsley flakes with different acetone:$H_2O$ solvents compositions (90:10, 70:30, 50:50, 30:70, 10:90, v/v). Samples were analyzed by HPLC with diode array detection (350 nm)

PLE technique are the flush volume, pressure, and static time (extraction time). Optimization of extraction time increases sample throughput and reduces the operation cost. The volume of solvent used for an extraction is determined by the flush volume setting. Optimization of the flush volume can reduce the amount of solvent used and waste generated per extraction. These three extraction parameters did not show any significant influence on extraction yield of phenolic compounds during the recent studies[24,25]. However, like the solid-to-solvent ratio, these parameters (flush volume, pressure, and static time) can also be optimized to increase sample throughput and reduce solvent waste generated during extraction, resulting in decreased cost per analysis.

## Conclusions

The results presented above clearly indicate that selection of modern techniques and appropriate optimization of extraction parameters (solvent composition, temperature, particle size, pressure, solid-to-solvent ratio, static time and number of extraction cycles) will allow chemists and chemical engineers to reduce the solvent consumed and waste generated during extraction of phytochemicals from different plant matrices. In addition, replacement of chlorinated and aromatic organic solvents with carbon dioxide, ethanol, water and other ‘green solvents’ will significantly reduce environmental pollution and health problems.

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