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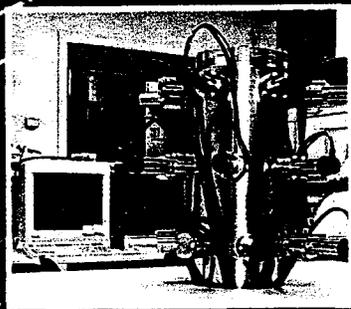
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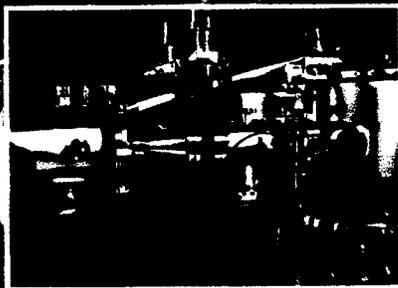
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SFE Starting to Make Headway in Food Analysis

Analytical labs are beginning to use SFE routinely and are expanding its extraction capabilities beyond those of current SC-CO₂ systems.

Analytical supercritical fluid extraction (SFE) has made considerable progress over the past decade in both theory and application. Ten years ago, it was thought that SFE would have its chief impact on environmental analysis, but most of its gains for industrial purposes have been in food and agricultural analysis, where it was first applied as a processing technique over 25 years ago.

In recent years, SFE has advanced well beyond the simple task of removing analytes from a sample matrix. SFE now encompasses cleanup, derivatization, and automation as standard features in many method protocols.

Today's analytical SFE systems incorporate many desirable features, including pressure, temperature, and flow rate; sample size; collection options; automated operation; cosolvent capability; and fluid delivery.

Modern SFE instrumentation is capable of offering extraction pressures of 10,000 psi, extraction temperatures approaching 200°C, and flow rates capable of processing samples from 10 to 25 g. Most SFE systems occupy a relatively small footprint on the lab bench. Some can be configured for portable or field use, or for use with such instruments as infrared spectrometers or liquid or gas chromatographs.

Perhaps the most welcome feature in today's instrumentation is automated operation. The trend has been toward sequential extraction, although parallel



Automated supercritical fluid extraction (ISCO Model 3560) is used for rapid analysis of fat and cholesterol in foods.

processing of samples is also practiced by multiplexing extraction units with a common fluid pumping source. Most systems can deliver cosolvents and recently have been altered to deliver liquids or subcritical fluids at elevated pressures and temperatures, even though users must still feed extractor units via single cylinders of compressed gas.

Getting the fat out

The most significant application of SFE in food analysis is in the determination of fat and associated nutrients. A considerable literature has been generated on methods for determining fat in foods and associated materials by SFE,

including techniques that determine fat by gravimetric assay as well as fatty acid distribution as required by the Nutritional Labeling & Education Act.

Some of the problems that plague traditional fat determination methods using organic solvents are also present in SFE methods, namely that all extraction of fat can be matrix dependent, particularly when it is bound through interaction with carbohydrate or protein moieties.

For example, SFEs of fat from meats, candies, and oilseeds can be conveniently accomplished by the methods generated to date, whereas it has proven difficult to determine SFEs of certain bakery products and pet foods due to inter-



molecular associations. The situation can be improved by the judicious use of cosolvents or by careful sample preparation prior to extraction.

Despite these reservations, SFE is being increasingly incorporated by institutions and regulatory agencies for fat and nutrient analysis. By substituting SFE, substantial reductions if not total elimination in the use of organic solvents in the analytical laboratory can be achieved. This has been aided by the automation of analytical SFE in formats ranging from 24 to 44 carousel-type extractors to parallel extractors providing integration of gravimetry with SFE. Aided by equivalence and collaborative studies, the future looks bright for utilizing SFE for the everyday analysis of fat levels in foods.

Alternatives to CO₂

CO₂ has been the major extraction fluid utilized in analytical SFE to date, but alternative fluids are being investigated for specific uses. This trend involves far more than the use of organic cosolvents as extraction aids, but the incorporation of both new and traditional entities in both their supercritical, subcritical, and pressurized liquid states.

For example, studies in our laboratory have shown that fluoroform (HCF₃) can extract pesticides selectively from food matrices and yield an extract with 100-fold less fat. This fluid has been further exploited by L.T. Tay-

lor and colleagues for the extraction of pesticide and drug moieties from biological and environmental matrices; however, its use may be limited by supply, price, and possible environmental controls.

Similar concerns also accompany new fluorocarbon-based fluids, such as Freon-22, which has been utilized by S.B. Hawthorne et al. for extracting environmental pollutants.

Today, less concern is voiced about maintaining a one-phase fluid system during the extraction; the prevailing philosophy is "If it works, use it." The authors have found that by starting with an organic solvent—a solvent that you want to replace, such as methylene chloride—and gradually replacing it with supercritical carbon dioxide (SC-CO₂), useful SFE and SFC can be accomplished. Similarly, S.V. Olesik has shown the merit of "enhanced fluidity" extractions, in which the presence of a supercritical fluid enhances the extraction performance of a traditional solvent.

When the traditional cosolvent approach is used in SFE, automated instrumentation aids considerably in screening for the appropriate cosolvent or blend of solvents. This has recently been used in our laboratory for optimizing the extraction of mycotoxins from agricultural matrices.

Even water in its subcritical state is

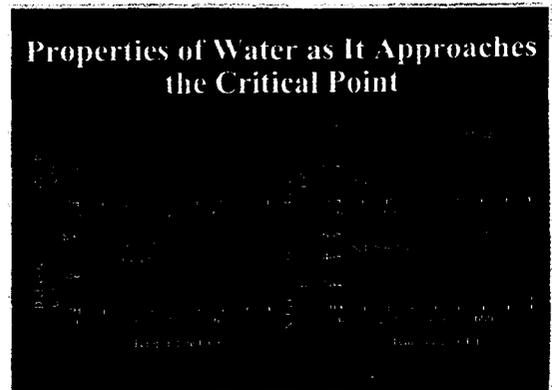
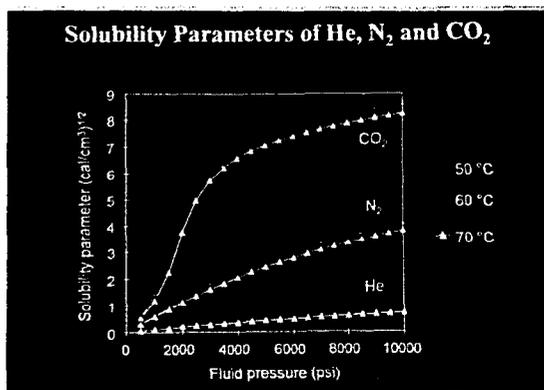
being exploited to extract polycyclic aromatic hydrocarbons/polychlorinated biphenyls from soil samples and pesticides from fruits and vegetables. Under these conditions, water must be regarded as a hot, pressurized fluid having a very low dielectric constant, thereby giving it a propensity for dissolving analytes normally extracted by nonpolar solvents. Thus, two fluids at extreme ends of the solvent polarity spectrum, CO₂ and H₂O, can be made complementary for providing extractions that are both versatile and environmentally benign.

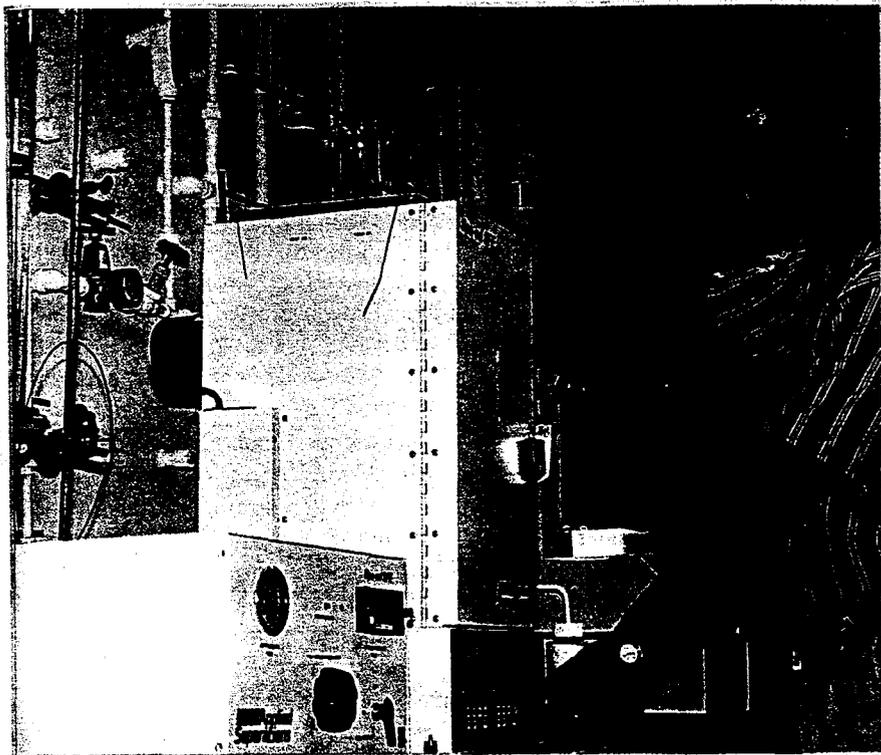
Integrating cleanup methods

Early in its development, it was recognized that SFE did not always yield extracts free from common interferences (such as fat in the case of food analysis). This has given rise to a number of novel methods involving the integration of cleanup methodology within or in sequence with the SFE step.

The fractionation of extracts can partially be accomplished on the basis of changing the extraction fluid's density, over the time of the SFE, or using the respective threshold density differences between the analytes and interfering components. However, in modern analytical SFE it has become more common to perform this differentiation between the targeted analytes and the interfering materials by using sorbent media, or by changing the nature of the extracting

Solubility of SC-CO₂ rises abruptly with pressures at less than 2000 psi (near right). SC-water exhibits unusual properties as a function of temperature and pressure (far right).





Modified Applied Separations' "Spe-ed SFE" unit is used for supercritical water extractions and reactions.

volatility parameter of SC-CO₂ rises abruptly with pressure under 2000 psi; control of coextractives is compromised under these conditions. However, fluids such as nitrogen or helium exhibit much less solvent power as a function of pressure and can be mixed with SC-CO₂ to reduce its solvent power as the pressure is increased. This can result in selective SFE of the target analytes while minimizing the carryover of lipid coextractives.

For example, a 30% N₂/70% CO₂ mixture results in the extraction of only 3-8 mg/ml of fatty coextractives while assuring 85% or greater recovery of the target analytes. This has been applied to yield extracts containing organochlorine and -phosphorus pesticides that are amenable for direct injection into a gas chromatograph.

Reactions of significance

Reaction chemistry in supercritical fluids offers many inherent advantages, including improvements in mass transfer, control of reactant/product solubilities, lower reaction temperatures, and control of the reaction rate and product distribution.

Many types of reactions have been performed in supercritical fluid media—enzymatic, polymerizations, conversions in SC-H₂O, heterogeneous catalysis, pyrolytic, and photolytic.

A growing number of applications have been developed in which reactions have proven of value in analytical SFE. In most of the applications, the main objective has been to increase the volatility of the target analytes, or to synthesize a known derivative, in preparation for the final analytical determination. The diversity of analytes

fluid.

The use of sorbents inside the extraction cell was first demonstrated in our laboratory for the cleanup of coextracted fat from pesticides on extracts from meat samples. This gave rise to a number of novel systems, some of which became commercialized, integrating solid phase extraction (SPE) both with and after the SFE step. The extract can be removed from the extraction cell and deposited on a sorbent cartridge, or the separation can be performed

on a sorbent that is segmented in the extraction cell. The alumina noted in the tandem off-line and in-line trapping modes can also be used to trap such nonpolar coextractives as fat, thereby allowing elution of the target analytes (pesticides or PCBs) for direct analysis. R.J. Maxwell and associates have used combinations of these cleanup/trapping modes for performing sample cleanup.

Most sorbents that have been utilized in conjunction with SFE exhibit low polarity, i.e., they are frequently identical to adsorbents used in many normal phase

LC applications. They include alumina, silica, Florisil, celite, surface-modified silicas, desiccant materials, adsorbent-based extraction disks, and synthetic resins/foams.

One SFE/sorbent-based cleanup technique deserves special mention since it can be applied readily for sam-

ple cleanup of extracts from fat-laden biological tissue. This concept, first investigated and characterized as "inverse" SFE by one of the authors, involves choosing

an adsorbent and extraction conditions that allow for the trapping of the target analytes within the extraction cell, while the lipid moieties are eluting in SC-CO₂. This inverts the usual SFE procedure, allowing the removal of then unwanted contaminating substances from the sample matrix via SFE. The technique has been exploited in applying analytical SFE for drug and food analysis.

The authors have developed a new sample cleanup technique using binary mixtures of supercritical fluid gases to moderate the solvent power of SC-CO₂. As shown in the attached figure, the sol-

Mixing nitrogen or helium with supercritical CO₂ can improve selective SFE while minimizing carryover of coextractives.

INSTRUMENTATION FOR FOOD TECHNOLOGY



that have been reacted under supercritical conditions—including polar analytes, polymers, and metal ions—is impressive.

Due to our interest in determining nutritional levels of fat in foodstuffs, we at NCAUR have become familiar with the enzymatic (lipase)-based catalysis of fats/oils to form fatty acid methyl esters (FAMES) in SC-CO₂/methanol mixtures after SFE. The technique and conditions for effecting this transformation arose from our scaled-up synthesis experiments to produce seed oil-based derivatives having industrial utility, such as biodiesel.

Using a lipase derived from the organism *Candida antarctica* (Novozym 435) supported on polyacrylamide resin, we have been able to transform triglyceride-based fats into their corresponding FAMES by sequentially extracting the lipids from food matrices, followed by reaction under supercritical fluid conditions in the presence of the Novozym 435 contained in the extraction cell. This extraction-reaction sequence works equally well for associated lipid species, such as cholesteryl esters and phospholipids, as demonstrated by J.M. Snyder et al.

A highly automated way of performing the above derivatization is shown in the attached diagram, where the scheme has been integrated into one of

the sequential SFE units. The extraction takes place in the initial part of the extraction cell using an SC-CO₂/methanol mixture while FAMES formation is conducted over the lipase bed downstream in the cell. The derivatized extract is transferred to a sorbent-based analyte trap and washed automatically with about 1 ml of solvent into a collection vial, which is then transferred via robotic arm to the autoinjector of a gas chromatograph.

This provides total unattended overnight analysis and has been used not only for fat analysis, but for evaluating the activity of different enzymes in the presence of SC-CO₂. The main objective of the procedure, however, is to determine the types of fat—saturated vs. unsaturated—present in the lipid extract. Results from this method agree well with those determined by conventional solvent extraction, thus removing any ambiguity concerning the nature of the extract and the presence of nonlipid coextractives.

Coupled techniques

Traditionally, coupling implied linking in-line SFE with GC, SFC, GC-MS, or FTIR. Because these tandem techniques require sophisticated personnel to operate the equipment, however, traditional SFE coupling has fallen out of favor in recent years.

Coupled methodology today implies

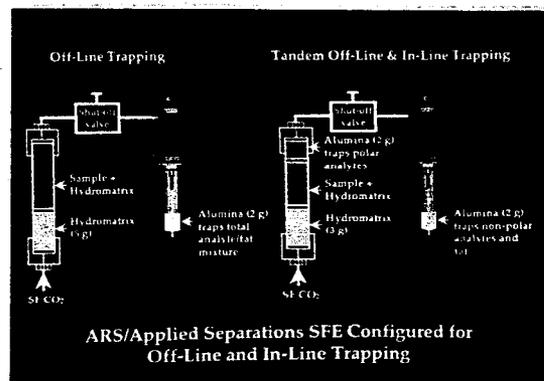
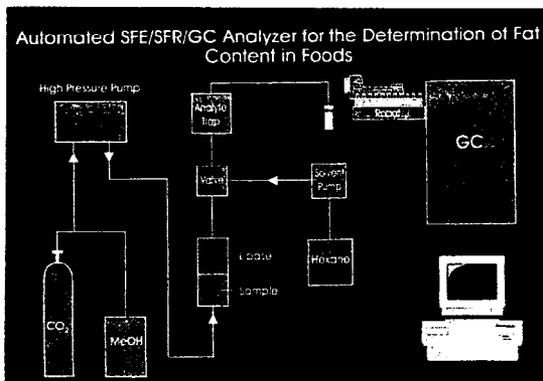
combining SFE off-line with an independent technique to yield a low-solvent approach for rapid analysis of target analytes—or it can imply a portable method that can provide a rapid analytical assay of food contamination in the field. Two of these low-solvent, environmentally compatible adjuvant techniques are enzyme immunoassay (EIA) and solid phase microextraction (SPME), both of which have been coupled with critical fluid extraction techniques.

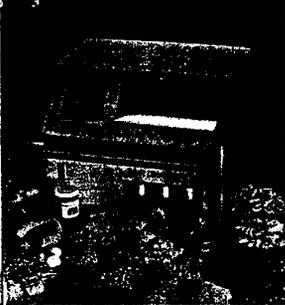
Studies in our laboratory have shown the feasibility and value of combining EIA with SFE for the analysis of pesticides and mycotoxins. For example, carbamate pesticide residues can be quantitatively determined in meats by a developed SFE/EIA procedure that requires only CO₂, water, and a small amount of methanol. This procedure eliminates many cleanup steps associated with the conventional chemical assay, including sophisticated post-column LC hydrolysis/derivatization fluorescence detection of the carbamate derivatives.

SPME techniques have been combined with subcritical water extraction

Overnight, unattended GC analyses of fatty acid samples can be performed using sequential SFE systems integrated into automated injection systems.

SFE techniques allow extracts to be removed from sorbent cartridges (left) or segmented extraction cells (right).





INSTRUMENTATION FOR **FOOD TECHNOLOGY**

for the detection of environmental pollutants, but in principle the technique can be applied to SFE extracts derived from foods, perhaps by using a coupled SC-CO₂-subcritical water extraction system. Recently, P.B. Liescheski amply illustrated the versatility of combining infrared spectroscopy off-line with SFE for determining such physical properties as iodine number and cis/trans fatty acid content of fats.

SFE horizons

Analytical SFE is taking on an increasingly important role in the analysis of foods, agricultural products, and botanical matrices. Several Federal laboratories are now using SFE routinely to screen for the presence of toxicants in different types

of food, and interlaboratory collaboratives are taking place.

Recently, the American Oil Chemists Society approved a standard Method 18 for determining oil in oilseeds.

For SFE to realize its full potential, however, will require more collaborative research and greater standardization of instrumentation to allow studies to be conducted conveniently.

—Jerry King and Zhouyao Zhang

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Note: The use of product names does not imply an endorsement by the USDA.

An expanded version of this article, with a full listing of technical references and additional information on supercritical water can be viewed on www.rdmag.com.

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