Table 2 Analysis of glycerides in a randomized fat sample

Methods of analysis	*MG*	*DG*	*TG*	*Time of analysis*
SFC-FID	0.2	9.6	90.1	25 min
GC-FID	0.1	6.9	92.9	30 min
HPLC-FID		13.5	86.5	1 h
HPLC-ELSD		8.0	92.0	30 min
LC-silica column	1.0	7.7	93.1	2 h
TLC	2.0	11.0	87.0	30 min

MG, Monoglyceride; DG, diglyceride; TG, triglyceride.

a randomized fat sample using six different analysis methods. The results given in Table 2 suggest that SFC-FID analysis yields comparable data for an equivalent analysis time to that obtained using the GC-FID and HPLC-ELSD methods. However, the SFC method does not require the time and effort for sample preparation associated with the alternative techniques and, in addition, saves on the cost of solvents and chemical reagents. A further illustration of the cost- and time-saving advantages of SFC is noted by its ability to monitor free and methylated fatty acids, thereby providing a reasonably quick and accurate assay for these compounds in foodstuffs to support nutritional analysis claims and the detection of frying oil deterioration as a function on time.

Preparative or production scale SFC is now being used as a separation technique in the food industry. Fractionation and isolation of higher value food components, such as tocopherols and phospholipids, or the ω-fatty acids/esters from fish oils, have been cited in the literature. Recently, a production plant for the separation of fish oil ethyl esters has been constructed in Spain to produce $\geq 95\%$ pure polyunsaturated fatty acids for the nutraceutical market. The basic separation design of this production scale plant is based on chromatographic fractionations initially developed using analytical scale packed SFC columns.

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Supercritical Fluid Extraction

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In the food industry, supercritical fluid extraction (SFE) is currently being used in a number of areas, shown in **Table 1**. The most attractive features of SFE in food processing is the fact that separation can be carried out at relatively low temperatures (40–60°C) using benign solvents. The solvent most widely used thus far is carbon dioxide, which is inexpensive, nontoxic, nonflammable, easily recoverable, and nonpolluting. The solubility and selectivity properties of SC-CO_2 has been compared with hexane. While both are nonpolar solvents, the selectivity of SC-CO_2 is enhanced in the presence of modifiers (entrainers). For example, in the absence of such polar modifiers as water and ethanol, SC-CO_2 alone is a poor solvent for extraction of caffeine from coffee beans or nicotine from tobacco. While the selectivity of SC-CO_2 and the mechanism of modifier action are not completely understood, studies in the area of supercritical fluid chromatography (SFC) have indicated that Lewis acid–base pairing, induced-dipole interactions

Table 1 Selected applications of supercritical fluid extraction in food processing

Process	*Commercial manufacturers*	*Literature source*
Coffee decaffeination	KaffeHAG AG, Germany; General Foods, Texas USA; SKW-Trotsberg, Pozzillo, Italy	Zosel (1978) Williams (1981)
Hops and spices extraction	SKW-Trotsburg, Munchmuenstar, Germany; Paul and While Beigat, UK; Pfizer, Sydney, Nerbraska; J.I. Hass, Yakima, Washington	Hubert and Vitzthum (1978) Vollbrecht (1982)
Flavours and fragrances	Flavax GmbH, Rehlingen Germany; Canilli Albert and Louis, Grasse, France	Calame and Steiner (1982) Caragay and Little (1981)
Vegetable oils and fatty acids	Mohi Oil Mills, Japan; Marbert GmbH, Dusseldorf, Germany	Stahl *et al.* (1980) Friedreich (1984)

and hydrogen bonding play an important role in determining the selectivity of SC-CO_2.

The principal disadvantage of SFE is that relatively high pressures (typically 50–100 atm or more) are required. Even though the energy savings make SFE attractive, the initial capital cost of high-pressure equipment overrides these considerations, especially at current energy prices. While the overall cost of SFE is dictated by such other factors as volume, price and the continuous or batch nature of the process, economic considerations have slowed its commercialization. Generally, SFE is best suited for difficult separations, not attainable by conventional processes. In situations where SFE can produce a new product or when environmental or regulatory concerns make its use more attractive, the application may more than justify the cost. The hazards of high pressure and the use of flammable solvents are also perceived unfavourably by many not experienced in these areas. While common in the petroleum industry, the food industry also uses a number of processes like homogenization, extrusion and compression routinely and thus should be able to deal with moderate pressures.

Another frequently overlooked problem associated with SFE is patent infringement. There are over one hundred patents on SFE of biomaterials in the United States alone. A potential user of these processes is likely to be faced with the involved task of determining if patent infringements exist or identify sources of legitimate licensing agreements. While SFE developments are growing globally, further research is needed both in terms of fundamental studies and applications.

The feasibility of extraction of a number of food materials using supercritical fluids has been investigated over the past two decades. In particular, much activity has focussed on extracting and refining fats, oils and their derivatives. The equilibrium solubility values of some of these are shown in **Table 2**. A number of advantages have been cited for the use of SFE in the processing of food-grade fats and oils from both animal and plant sources. These include:

- Low temperature processing reduces degradation of temperature and oxygen-sensitive components.
- Both extract and raffinate are free of solvent and can be used in food.
- Extraction and fractionation into various cuts of different physicochemical properties can be performed simultaneously.

Table 2 Solubilities of selected food materials in supercritical fluids

Food material	*SC solvent*	*Solubility (%wt/wt)*	*Extraction (°C)*	*Conditions (bar)*	*Co-solvent*	*Reference*
Beta-carotene	Ethylene	0.17	50	374	–	Chang and Randolph (1989)
		0.23	70	374	–	
Cholesterol	CO_2	0.33	60	270	–	Wong and Johnston (1986)
		0.37	60	270	MeOH	
Coumarin	CO_2	0.7	40	100	–	King and Friedrick (1990)
Butterfat	CO_2	2.2	40	248	–	Yu *et al.* (1992)
Palm oil triglycerides		1.1*	75	300	–	Brunner (1994)
	Ethylene	2.2*	70	300	–	
Soybean triglycerides	CO_2	0.6*	50	300	–	Friedrich (1984)
		3.0*	50	600	–	
Hop extract	CO_2	9.4	80	400	–	Stahl *et al.* (1987)
Evening primrose oil	CO_2	8.0	40	300	–	Lee *et al.* (1994)

*Estimated from graphs.

- A continuous and large-scale process can be economically competitive to hexane-based operations.

Exploiting the commonality of high pressure between supercritical fluid and extrusion processing operations, a hybrid unit operation called supercritical fluid extrusion (SCFX) has been recently developed. This new process permits generation of microcellular structure at low temperature by using SC-CO_2 as a blowing agent instead of steam to puff the extrudate, thus decoupling the conventional dual role of water, which otherwise serves both as a blowing agent as well as a plasticizer. The use of supercritical fluid also permits deposition of solute into the extrudate matrix.

Significant progress has also been made in the analysis of food and related materials using SFE with SFC. Sample preparation for analysis often requries orders of magnitude more time than the analysis itself and the use of supercritical fluids obviates the need for hazardous organic solvents with no additional treatment prior to identification of the analyte by other techniques such as GC, GC-MS, FTIR, etc. The solubility of lipid-like materials in SC-CO_2 ranges from 1 to 30 wt%, depending on the density of the fluid used, and therefore, SFE has become a method of choice for rapid extraction of fats and oils from a variety of food matrices such as animal, vegetable, grain and seafood products. Other successful applications include extraction of fat-soluble vitamins, pesticides, sterols, and fatty acids. As an analytical tool, SFC has also made significant progress over the past decades but has yet to prove its superiority over the more conventional techniques.

See also: **II/Extraction:** Supercritical Fluid Extraction. **III/Food Technology:** Supercritical Fluid Chromatography. **On-Line Sample Preparation: Supercritical Fluid Extraction.**

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FORENSIC SCIENCES

Capillary Electrophoresis

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Several slab electrophoretic techniques have frequently been used to discriminate between red cell enzyme markers as a means of identification in criminal cases over many years. In 1991 capillary electrophoresis (CE) was introduced to forensic analysis. The separation of bulk heroin, heroin impurities and degradation products using micellar electrokinetic capillary chromatography (MEKC), the determination of drugs of abuse in urine and also the determination of benzodiazepines and sulfonamides in urine by CE–mass spectrometry were described in the same