REVIEWS

Extraction of Natural Substances with Dense Gases

Egon Stahl[1] and Karl Werner Quirin[1]

Abstract: An overview of the solvent power of dense gases is given. The properties of these novel solvents are described and the specific advantages of carbon dioxide discussed. New qualitative and quantitative procedures for the rapid determination of solubilities are described for several interesting classes of natural substances. These include fatty oils, steroids, alkaloids, flavor and aroma substances, which documents the versatility of the new extraction procedure in many practical applications.

The solubility of low-volatile substances in dense gases is a well known phenomenon. The first experimental tests that were carried out over 100 years ago dealt with the solubility of inorganic salts in supercritical ethanol (1). Whereas the practical application of this phenomenon raised only little attention in the beginning, its importance for the formation of minerals under hydrothermal conditions (2, 3) and the migration of petroleum in the earth crust under the influence of gases (4, 5) was early recognized. Today, the practical applications not only extend to the cultivation of synthetic crystals (6) and the recovery of viscous dead-oil (7, 8), but also to novel procedures for chemical separations. Of particular interest here are the extraction of natural substances and their raffination.

Gases with critical temperatures (T_c) near the working temperature of the separation method are used as solvents. By applying high pressures it is possible to produce liquid-like densities that are a prerequisite for the solvent capacity of the gas. Dense gases, therefore, allow one to selectively adapt the solvent properties to the problem to be solved by varying the parameters pressure and temperature. Further outstanding characteristics of dense gases, when compared to classical solvents, include favorable transport behavior, i.e. low dynamic viscosity and high diffusion coefficient despite its liquid-like density (9, 10), and simple separation of solute and solvent (Table I). Gases that can be used in the extraction of natural substances are compiled in Table I according to their increasing critical temperatures. They represent typically lipophilic solvents. Carbon dioxide takes up a special position as it is physiologically unobjectionable and without environmental stress problems. CO_2 is available in large amounts at low prices with little dependence on the petrochemical industry. It is nonflammable and nonexplosive and, thus, offers many requisites for laboratory experiments and technical applications.

Table I. Physico-Chemical Data of Various Gases Usable for Extraction

Gas	bp (1 bar) (°C)	T_c (°C)	p_c (bar)	ϱ_c (g/cm³)
Ethylene	−103.7	9.5	50	0.20
Trifluoromethane	− 81.4	28.8	39	0.58
Carbon dioxide	− 78 (subl.)	31.3	73	0.448
Ethane	− 88	32.4	48	0.201
Nitrous oxide	− 89	36.5	71	0.457
Propylene	− 47.4	91.8	45	0.22
Propane	− 44.5	96.8	42	0.220
Difluorodichloromethane	− 29.8	111.8	39	0.558

Substance Separation and Extraction with Dense Gases

General Survey

Substance separations are important in various fields, and therefore, attempts to use dense gas in novel separation procedures are numerous. Many applications have only been tested on a laboratory scale. However, the industry is also interested in this new technology as can be seen by the high number of patents filed within the last years. The existing propositions can be classified into various groups.

Classical solvents are used at high temperatures under supercritical conditions in the extraction of *coal, tar sands* and *peat* (11, 12) with the aim to liquefy the largest possible part of the raw material by pyrolysis/extraction. The fractionation of already liquid crude oil products and residues is yet another problem to be solved by this approach (13–16). A similar task lies in the extraction of *wood* to obtain liquid fuels (17, 18) or the separation of special chemicals under milder conditions (19, 20).

The extraction and raffination of *vegetable oils* represents another extensive area of application (21–24) where the use of dense carbon dioxide can replace several steps of classical procedures which largely reduces the total expenditure for the edible end product.

Mainly in the patent literature, there are a series of publications concerning the detoxification of *tea* (25), *coffee* (26) and *tobacco* (27) with unique methods differing in the details of the process technology. The extraction of *flavors* (28, 29), *spices* and *volatile oils* is of special interest to the food sector. CO_2 allows one to obtain solvent-free concentrates of highly appreciated sensory qualities without the application of high temperatures.

The good solubility of these substances in dense CO_2 and their high market value are a strong incentive. The patent literature (30–33) is correspondingly extensive. Furthermore, there are a number of *special applications,* such as the separation of alcohol-water-mixtures (34) and the regeneration of adsorbents (35).

Micro-Extraction with Dense Gases

In comparison to classical solvent extraction, the extraction with dense gases is more difficult and the set of parameters more complex. For preliminary tests a simple procedure has, therefore, been developed that requires only small amounts of

[1] Department of Pharmacognosy and Analytical Phytochemistry, University of Saarland, D-6600 Saarbrücken, W. Germany

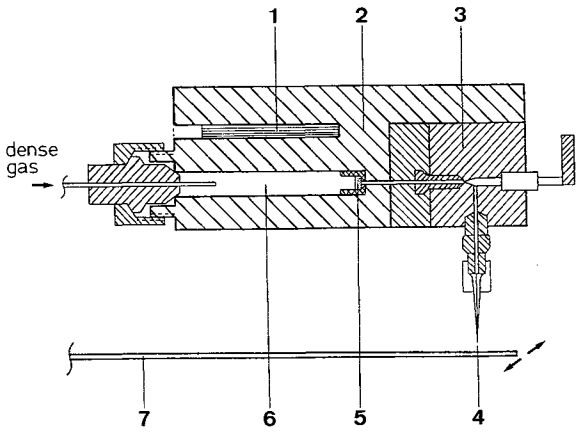

Fig. 1 Cross section of the micro-autoclave for dense gas extraction and transfer of the solute onto a chromatoplate. 1 heating element, 2 brass block, 3 valve, 4 glass capillary, 5 filter, 6 extraction chamber, 7 thin-layer plate.

material and informs rapidly on the extractability of substances as a function of pressure and temperature. The method is based on the direct coupling of a micro-extraction apparatus with thin-layer chromatography (36) (Fig. 1).

A certain amount of dense gas is guided under defined conditions through a microautoclave (2 ml) that contains the powdered plant material or the pure substance adsorbed on an inert carrier material. The gas – corresponding to its condition – dissolves part of the substances and is expanded through a fine glass capillary (∅ 10 μm). The dissolved substances precipitate and are transferred by the fine gas stream as a band onto the back- and forth-moving thin-layer plate. Several extracts are applied side by side under different conditions. After chromatographic development and detection on plate, the extraction behavior, e. g. of various lipids from animal or plant tissues (3), can be assessed.

Numerous natural compounds and other substances were examined with this method for their solubilities in CO_2 (38). The following *rules of thumb* apply to the extractability:

1. Easily extractable in the pressure region up to 300 bar are lipophilic substances with a molar mass of ≤ 400, e. g. hydrocarbons, ethers, esters, ketones and similar compounds.
2. The introduction of polar functional groups, e. g. -OH, -COOH, decreases the extractability or renders it impossible.
3. Polar substances, such as sugars and glycosides, amino acids, phospholipids and polymeric compounds, such as proteins, cellulose, polyterpenes, and plastics, are non-extractable. Non-polar oligomers are soluble to a small extent.
4. Water is barely soluble in liquefied carbon dioxide, while in supercritical CO_2 it is increasingly soluble with increasing temperature.
5. Fractionations are possible if there are differences in molar mass, vapor pressure or polarity of the substances.

The class of steroids provides an impressive example for the relationship among chemical structure and solubility. A study of 35 steroids at 40°C in supercritical carbon dioxide (39) shows the strict dependence of solubility on nature and number of substituents on the tetracyclic steroid structure. Compounds with one hydroxyl group, all sterols, are extracted starting already at 80 bar, those with two hydroxyl groups at 120 and those with three hydroxyl groups above 150 bar. The strongest influence is seen with the carboxyl group, e. g. in bile acids; only desoxycholic acid is soluble above 200 bar.

Quantitative Solubility Determinations

Quantitative solubility determinations can be carried out according to static methods that require a phase equilibrium apparatus (40) or according to the dynamic method. The latter more practical technique is based on the percolation principle, and it yields solubility values that are close to the equilibrium values (41). The simple method of micro-extraction with expansion through a glass capillary is also suitable for quantitative measurements, if the thin-layer plate is replaced by a trap, e. g. a glass funnel containing an adsorbent and connected to a flow meter in series. This procedure allows a quantitative recovery of the extract as well as an exact account of the gas consumed in the test. The calculation of solubility in mg/Nl* or per cent by weight by the data obtained requires no knowledge of the pVT-data of the dense gas.

With this method, we have examined the solubilities of the *opium alkaloids,* codein, thebaine, noscapine, papaverine and morphine, in the dense gases CO_2, N_2O and CHF_3 (42). The extracted material was collected in a glass funnel, eluted in a small measuring flask, and the content was then determined photometrically. It was found that nitrous oxide as solvent is superior to the isosteric carbon dioxide with the same selectivity with respect to the molar mass and the polarity of the alkaloids. Supercritical trifluoromethane exhibits a better solvent power, but at the cost of selectivity.

Similar to the alkaloids, the single-substituted *steroids* only reach solubilities between 0.8 and 1.5 mg/Nl CO_2 at 200 bar and 40° C. Each further substituent substantially decreases this value. Other gases, such as sulphur hexafluoride, ethylene and trifluoromethane, exhibit less solvent capacity for steroids than carbon dioxide despite the fact that some of these display higher densities and dielectric constants.

Solubility is dramatically increased by the simultaneous application of high pressures and temperatures; for example, the solubility of sitosterol in CO_2 at 700 bar and 80°C increases to over 20 mg/Nl (43).

The described micro-procedure is less suited for the solubility determination of compounds that are difficult to detect or of substance mixtures, e. g. of a natural plant oil. In this case, the dynamic method offers the possibility of carrying out the same procedure on a preparative scale where the extracted substances can be determined by weighing. For this purpose we have developed a new apparatus with a 40 ml extraction volume. It allows measurements up to 3000 bar because of a two-step compression. However, on this scale, it proves to be most difficult to control and maintain defined conditions in the dynamic system.

This apparatus enabled us for the first time to examine the solubility of *soy bean oil* (44) as a model for triglycerides and of *jojoba oil* (45) as a model for wax esters in dense CO_2 over a large pressure and temperature range. The solubility isotherms – in the examined temperature interval – show an intersecting point at 280 bar for soy bean oil (Fig. 2) and at 250 bar for jojoba oil. Here, the temperature does not exhibit a marked influence on the solubility, whereas with increasing temperature the solubility increases in the higher pressure range and decreases in the lower one. In addition, the measurements show that in supercritical carbon dioxide increasing the pressure to 700 and 1000 bar continously raises the solubility of jojoba oil and soy bean oil, respectively, to a maximum and

*1 Nl = 1000 cm^3 at 1 bar, 0° C = 1.977 g CO_2

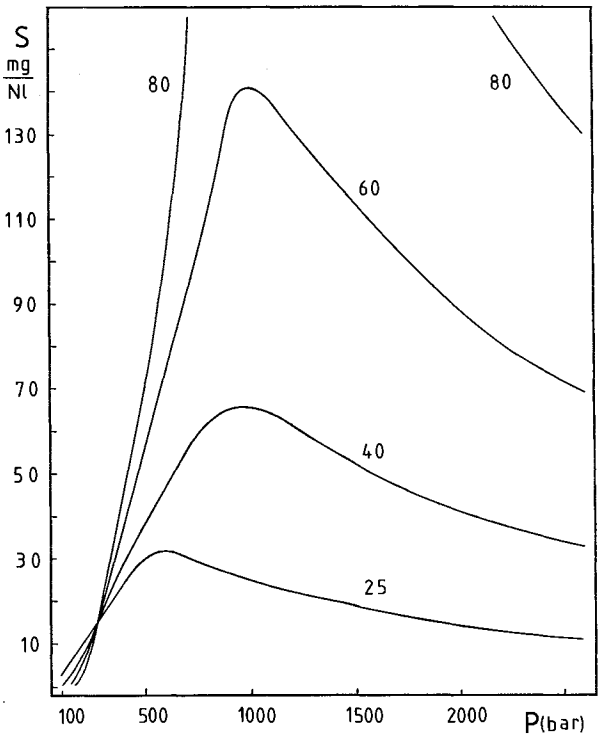

Fig. 2 Solubility isotherms – at various temperatures (°C) – of soybean oil in liquefied and supercritical carbon dioxide as a function of pressure.

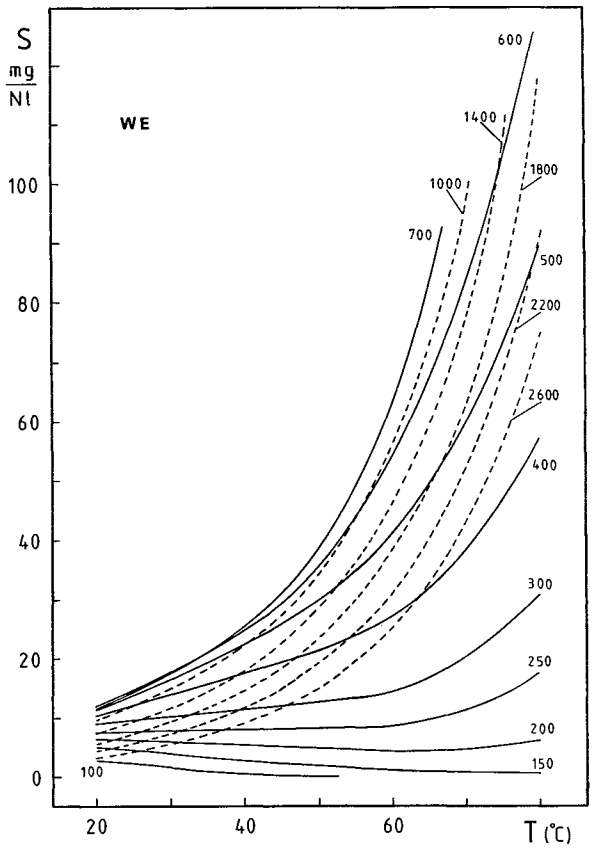

Fig. 3 Solubility isobars – at various pressures (bar) – of jojoba oil in liquefied and supercritical carbon dioxide as a function of temperature.

then leads to a less pronounced decrease in solubility. The increase is especially marked above 60° C, and higher temperatures, in the pressure range of the solubility maximum, probably cause complete miscibility. It appears that the phase behavior of the triglycerides and wax esters deviates from that of other substances that show a flattening of solubility already at 200 bar and, thus, possess solubility isotherms similar to the density isotherms of the gas.

Fig. 3 shows the solubility data of jojoba oil, presented as isobars. One can clearly see that the solubility of wax esters is relatively small in liquefied carbon dioxide below 31° C and only slightly influenced by pressure, whereas above 50° C, the solving capacity of CO_2 in the pressure range of 400 to 700 bar reaches high values that are suitable for the preparative extraction of oil seeds. Moreover, the steep increase of the isobars at high pressures allows a simple and economical recovery of dissolved substances merely by decreasing the temperature.

Preparative Substance Separation

The micro-extraction apparatus coupled with thin-layer chromatography provides valuable information on the choice of the extraction parameters at the preparative scale. Selective separations may be aimed at, and they may also be optimized if there are additional quantitative solubility data of the substances of interest. Suitable approaches to experimental planning are discussed in the literature (46, 47). Numerous natural products have been separated from plant tissues in an extraction apparatus that – depending on the problem to be solved – permits the installation of autoclaves with up to 5 l capacity and provides the possibility of solute precipitation in two stages (Fig. 4).

For the extraction of *alkaloids* from plant material (48) it must be considered that a reasonable solubility, observed during the testing of pure substances, does not always correspond to a fast and quantitative extraction of these substances.

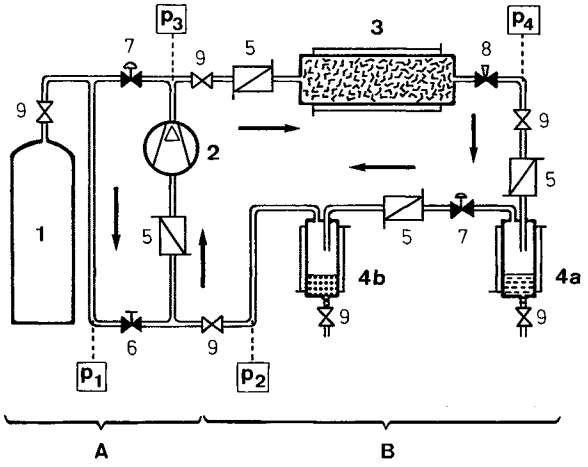

Fig. 4 Scheme of an extraction apparatus with two step solute recovery. A) Pressure generation and regulation, B) Extraction and separation.
1 CO_2 tank, 2 compressor, 3 extraction autoclave, 4a, 4b separation vessels, 5 heat exchanger, 6 reducing valve, 7 back pressure regulator, 8 micrometer valve, 9 shut-off valves.
p1: pressure in CO_2 tank, p2 ≤ p1: input pressure of compressor and pressure in separator 4b, p3 ≥ p2: extraction pressure, p4: pressure in separator 4a.

Depending on the drug and the basicity of the alkaloids, these can be completely bound to the plant acids as well as to tanning agents. In this form they are not readily extractable by lipophilic solvents. Pretreatment of the plant material with alkaline normally facilitates the liberation of the alkaloid bases. A certain water content in the carrier material can promote the selective alkaloid extraction.

Essential oils are highly soluble in dense carbon dioxide, as they are low molecular lipophilic substances with relatively high vapor pressure. The problem is the choice of extraction conditions that prevent the coextraction of undesirable substances that may also be present. On the other hand, the separation conditions must be carefully chosen to recover these volatile substances as completely as possible. Experiments were, therefore, carried out to minimize residual solubility in the partly expanded gas (49, 50).

Other procedures were designed to obtain thermally labile compounds genuinely contained in plant tissues that can be separated only as rearranged or secondary products by conventional methods. Thus, we succeeded in isolating the sensitive flavor and the thermally labile proazulenes (e. g. matricine) from *chamomile flowers* (51). The labile acoragermacrones and other sesquiterpenes can be extracted undecomposed from *calamus rhizomes,* whereas decomposing to shyobunones occurs by the usual steam distillation (52). The thermally labile artabsin which reacts under the conditions of steam distillation to dihydrochamazulenes can be extracted in the intact form from *wormwood herb* (53). The essential oil from wormwood herb which contains the toxic ß-thujone can be separated completely and selectively with CO_2 of lower density (e. g. 40°C/100 bar). Under these conditions, artabsin is only partly extracted and the content of the main bitter ingredient absinthin which determines the value of the herb remains practically unchanged (54). There is no value-decrease of the herbs by thermal stress and also no solvent residue, so that the refined carrier material can be used without any objection for pharmaceutical preparations and for the production of bitter liquors and wines.

Because of the high selectivity of the dense gas procedure, extracts can be recovered which are of higher content in active ingredients than conventionally obtained products. For instance, *pyrethrum flowers* yield chlorophyll free extracts which contain more than 60 per cent of the insecticidally active pyrethrins (55), and CO_2 extraction of *valerian roots* yields concentrates with a high content of sedatively active ingredients (56).

Extraction of several *spices* yielded essential oils which were superior in their sensory quality to those obtained by steam distillation. We were able to obtain a good separation of the caraway extract into fatty and in essential oils by a three-stage solute precipitation (57). Moreover, liquefaction of the CO_2 succeeded in selectively separating coextracted leaf waxes from the essential oil (45).

It is also of interest that lipophilic *insecticides* such as DDT and HCH, can be removed quantitatively from contaminated carrier material by extraction with dense carbon dioxide already at 40° C and 100 bar. This procedure can be recommended for the detoxification of plants with polar, that is insoluble, active ingredients. With senna leaves and pods and with thorn apple leaves we were able to show that the content of the active ingredients, sennosides and tropa alkaloids, respectively, remain unchanged if treated in this way. They are even better extractable with water from the treated compared to the original plant material (58).

With respect to *fatty oils* the extraction with dense CO_2 proved its value in the recovery and fractionation of the oil from soy beans, sun flower seeds, rape seeds, cotton seeds (59), and from lupine seeds (60). In all cases yields were similar to those of classical solvent extraction, while the extracted oil is practically free of phospholipids (60, 61). A large portion of the free fatty acids as well as undesirable flavor and aroma substances can be removed by fractional separation which renders refining much easier or even unnecessary (62, 63). The seed residues contain proteins and are used as animal feed; they do not show any denaturation of the proteins (64, 65) and need not be freed of solvent residues.

Production of Oil-Free Lecithin by High Pressure Jet Extraction

High pressure extraction of liquid or viscous material is problematic as it is difficult to obtain an intensive and steady contact with the solvent. The situation becomes critical if the viscous product to be extracted consists of solid and liquid components, as is the case for raw lecithin, a suspension of solid phospholipids in liquid triglycerides. The removal of the triglycerides is presently carried out by treatment with acetone. Since a large part of the phospholipids is used in the food and pharma industries, there is substantial interest in alternative refining procedures with physiologically unobjectionable conditions that avoid any residue problems. Extraction with dense CO_2 offers itself as the method of choice because of the highly selective solubility of triglycerides and less polar lipids (Figs. 2 and 3) in relation to phospholipids. Most of the experiments so far were unsuccessful, as with increasing extraction of the liquid components the mass becomes increasingly stiff which prevents more and more the complete separation of the triglycerides.

We were able to solve this problem by developing a new high pressure jet technique that is schematically shown in Fig. 5 (66). The viscous starting material can easily be forced at sufficient rates through one (or more parallel) stainless steel capillaries of defined size (e. g. 0.2 mm internal diameter), which greatly enlarges the surface of the solute. A second capillary, only slightly larger, is placed concentrically over the exit of this substrate jet overlapping it to a certain extent. The dense gas enters parallel to the substrate through the narrow ring-shaped interstice and thereby achieves a high velocity. At the end of the solute feeding jet turbulences arise that provide an intensive mixing of the substrate with fresh solvent. A short distance is sufficient for complete extraction of the small mass particles formed. The raffinate, i. e. the deoiled lecithin, is collected in the first separator, and the extracted oil is transported further in the flow of dense gas and precipitated after expansion in a second collection vessel. We could thereby obtain a light-colored powdered pure lecithin of high quality in a single raffination step from the crude starting material. The described jet procedure is also suitable for other separation problems, for instance for the extraction of liquids, emulsions, and dispersions. In most cases a continuous operation is possible.

Continuous Deterpenation of Essential Oils

Citrus oils contain up to 90 per cent monoterpene hydrocarbons. As the oxygen containing oil components are the actual

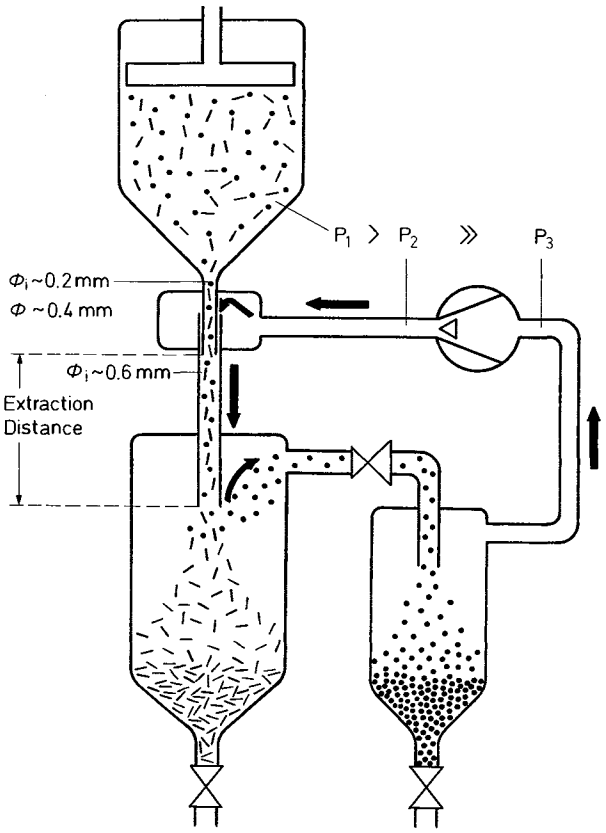

Fig. 5 Scheme of the continous high-pressure jet extraction of viscous masses.

flavor carriers and as the unstable hydrocarbons decrease the flavor quality, it is necessary to reduce the content in hydrocarbons to achieve improved oil quality. This is possible by counter-current extraction with supercritical CO_2 in a fractionation column already at 80 bar (49), which provides an alternative to the classical procedures that can lead to thermal stress or create residue problems. The higher volatility of the hydrocarbons as well as their lower polarities compared to the remaining oil components are exploited for the separation. The essential oil is fed continously into the middle of the column. Dense CO_2 is introduced at the bottom of the column, enriched with monoterpene hydrocarbons when ascending and is then removed at the top of the column. The refined essential oil contains little terpenes but all the coloring matter of the pressed starting material; it is collected at the base of the column through a valve. The oil is of high sensory quality and highly suited to aromatize and color drinks and liquors. Fig. 6 shows the capillary gas chromatogram of a bitter lemon oil (A) and of the fraction which is poor in terpenes (B) because of the removal of 92 per cent of the originally present terpenes by CO_2 extraction.

Conclusions and Outlook

The extraction with dense gases is a versatile separation procedure with many advantages over conventional methods, especially for the extraction of natural substances. The existing gap between recognized possibilities and industrial use can be explained by the high investment costs for high pressure plants and by their partly discontinous operation in the past. With progressing technological development, increasing cost for energy and classical solvents and with the increasingly strict food laws, the high pressure procedure raises more and more interest. The separation procedures with dense gases will, therefore, definitely enjoy a favorable development in the near future.

Acknowledgement

We thank the Deutsche Forschungsgemeinschaft and the Fonds Chemie for supporting our work.

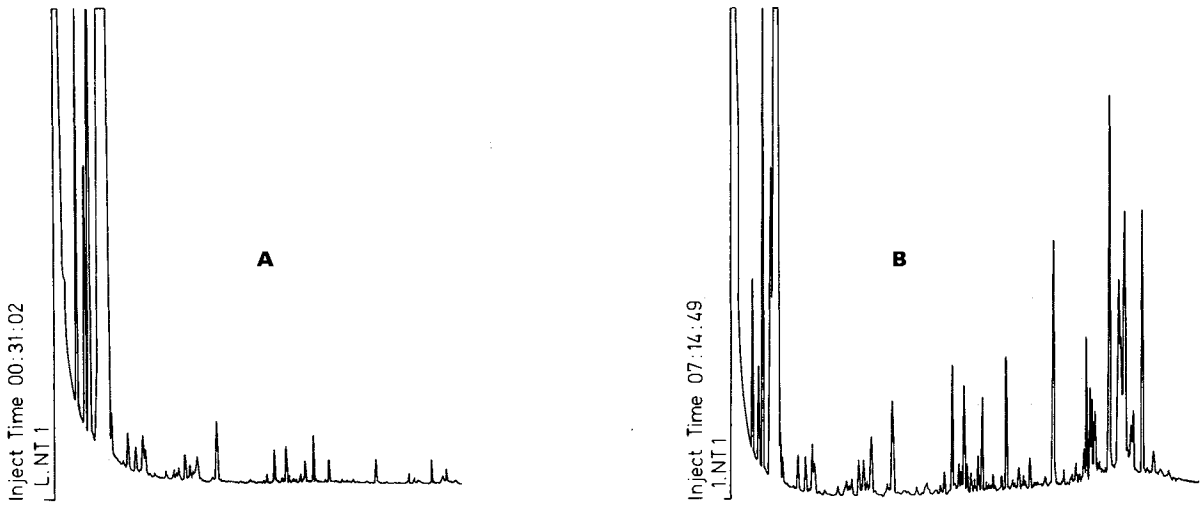

Fig. 6 Capillary gas chromatogram of a bitter lemon oil. A) Original, B) concentrated flavor fraction.

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Pyrazolopyrimidine Metabolism in Parasitic Protozoa

Buddy Ullman[1]

Abstract: The pyrazolopyrimidines are purine analogs that are cytotoxic toward and metabolized by several genera of parasitic protozoa, including the *Leishmania* and the *Trypanosoma*. Examples of pyrazolopyrimidines that are selectively metabolized by these parasites include allopurinol, allopurinol riboside, 4-thiopurinol, 4-thiopurinol riboside, and formycin B. These pathogenic protozoa are capable of efficient conversion of the pyrazolopyrimidines to the nucleotide level. The pyrazolopyrimidine metabolites which are isomers of inosine monophosphate are subsequently aminated and incorporated as the adenylate analog into RNA. Mammalian cells are incapable of these metabolic transformations. The sulfur containing pyrazolopyrimidines, however, are neither aminated nor incorporated into nucleic acid. The selective metabolism of the pyrazolopyrimidines by the intracellular metabolic machinery of the parasites of the Trypanosomatidae family offers a rational approach to the chemotherapy of the diseases caused by these pathogenic hemoflagellates.

[1] Department of Biochemistry University of Kentucky Medical Center Lexington, Kentucky 40536-0084, USA