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THE SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF SOME FOOD RELATED PRODUCTS

by

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ABSTRACT

The sc-CO_2 extraction of annato pigment from seed, piperine from black pepper corns and caffeine from coffee beans was shown to be feasible, yielding extracts comparable to those obtainable by solvent extraction.

A principal feature of the investigation was that it revealed the contribution of quite a few variables not normally considered to have a major influence on sc-CO_2 botanical extraction. One of these is the natural moisture and light oil content of the plant material that act like internal cosolvents influencing the solvent characteristics of sc-CO_2 in a similar way as an added external cosolvent adjusts the polarity of the fluid.

The extraction data were processed by linear regression analysis and goal seek statistics available in a commercial software package. It offered the possibility to predict the outcome of an extraction for a moderate change in one parameter while all others are kept constant. The regression fit, however, was not based on real process modelling but rather on an algebraic summation of the contribution of different variables, thus preventing statistical weighting to be applied to the different parameters.

The extractions were performed on both micro and pilot plant scale and thereby demonstrated the ability to upscale supercritical work.

The mechanism of botanical extraction by sc-CO_2 was shown to be principally governed by dissolution of a desired substance by virtue of the density and thus the solvent strength of the fluid and by the magnitude of the corresponding activation energy. This suggests that the extraction process is chemical in nature.

OPSOMMING

Dit kon aangetoon word dat sc-CO_2-ekstraksie van annatto-pigment uit saad, van piperien uit swart peperkorrels en van kaffeïen uit koffiebone uitvoerbaar is en dat die verkrygde ekstrakte vergelykbaar is met dié wat met oplosmiddelekstraksie verkry kan word.

'n Belangrike aspek van die ondersoek is dat dit die bydrae van 'n hele paar veranderlikes aan die lig gebring het wat na verwagting normaalweg nie 'n groot invloed op botaniese ekstraksie met sc-CO_2 sal hê nie. Een hiervan is die natuurlike vog- en olie-inhoud van die plantmateriaal wat as interne ko-oplosmiddels die oplosmiddeleienskappe van sc-CO_2 op 'n soortgelyke wyse beïnvloed as wat 'n ekstern toegevoegde oplosmiddel die polartiteit van die fluïed verander.

Die ekstraksiedata is verwerk deur lineêre-regressie-analise en optimaliserings-statistika wat in 'n kommersiële sagtewarepakket beskikbaar is. Dit het die moontlikheid gebied om die uitwerking van 'n matige verandering van een veranderlike op 'n ekstraksie te voorspel terwyl al die ander veranderlikes konstant gehou word. Die regressiepassing was egter nie op werklike prosesmodellering gebaseer nie maar eerder op 'n algebraïese sommering van die bydrae van verskillende veranderlikes. Gevolglik kon daar nie statistiese gewig aan die verskillende veranderlikes toegeken word nie.

Die ekstraksies is op sowel mikro- as loodsaanlegskaal uitgevoer, waardeur die moontlikheid om superkritieke werk op te skaal, gedemonstreer kon word.

Dit kon aangetoon word dat die meganisme van botaniese ekstraksie met sc-CO_2 hoofsaaklik berus op die oplos van 'n verlangde stof soos bepaal deur die digtheid en dus die oplosmiddelsterkte van die fluïed asook deur die ordegrootte van die ooreenstemmende aktiveringsenergie. Dit dui daarop dat die ekstraksieproses chemies van aard is.

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Chapter 0

A Brief Orientation

Ever since it was demonstrated in 1879 that potassium iodide could be dissolved in supercritical ethanol and then reprecipitated as a salt by reducing the pressure,[1] supercritical fluid technology has captured the interest of scientists and engineers alike. Many have tried to explain the phenomenon of the supercritical state, the unique behaviour of supercritical fluids and the mechanism of supercritical fluid extraction (SFE) with different theories, but the topic appears to be diverse and complex, still eluding researchers to this day.

Supercritical carbon dioxide (sc-CO_2) occurs in the region of the phase diagram above 73 atm and above 31°C. Although in many instances N_2O appears to be a better solvent than CO_2, it is a strong oxidising agent and cannot be used in many applications. This leaves CO_2 as the preferred substance to be used as a supercritical fluid, especially since it is inexpensive, non-toxic, inert, non-inflammable and readily available.

0.1 Botanical extraction - a topic worth investigating

Almost all supercritical fluid extraction (SFE) work done on natural products has been performed in such a way as to obtain an extract comparable to that obtained by conventional methods.[2] The driving force behind much of this work is legislation regarding the quality of food additives and the requirement to have botanical extracts free from hazardous solvent residues.[3]

sc-CO_2 is a solvent, much like water or any other solvent, that can dissolve certain substances. It differs from other solvents in that different properties pertaining to its polarity[4] are exhibited under different conditions of pressure, temperature and solutes present. These solutes, whether added deliberately, or forming part of the raw material being extracted, tend to modify the dissolving properties of the supercritical fluid, enabling it to become either more or less hydrophilic or hydrophobic. This tends to either decrease or increase its polarity as a solvent, thereby dissolving substances of different polarity from the plant matrix according to the rule "like dissolves like".

A study has been undertaken in which a selected variety of food related natural products were subjected to sc-CO_2 extraction using commercially available laboratory size and pilot plant scale supercritical fluid extractors. This dissertation reports on the outcome of this study.

0.2 Goal of this investigation

This dissertation deals with the sc-CO_2 extraction of annatto, piperine and caffeine as a selected few natural product ingredients to

(i) illustrate the capability of supercritical technology to produce quality botanical extracts;
(ii) optimise the conditions by which maximum yields of selected ingredients can be obtained;
(iii) gain more experience of and insight in manipulating the solvent strength and transport characteristics of sc-CO_2 to selectively extract specifically desired substances;
(iv) add to the knowledge of sc-CO_2 extraction, a field that is still largely unexplored and for which the possibilities and challenges of novel applications are only limited by the imagination of scientists;
(v) test the feasibility of implementing regression analysis and goal seek statistics to manipulate the conditions and outcome of sc-CO_2 extraction;
(vi) acquire a better scientific and practical understanding of sc-CO_2 based extraction which could render industrial application more attainable;
(vii) provide guidelines for future work in view of what was achieved in this investigation.

Ideally, engineers and scientists should have a universal solvent, in the form of a supercritical fluid, which can be modified to selectively extract a desired product. This, however, requires more to be understood about the principles of supercritical fluid extraction, a goal which was pursued by virtue of this investigation.

References

1 J B Hannay, J Hogarth, Proc. Royal Soc. (London) 29, 324 (1879).

2 H Brogle, "CO_2 as a solvent: its properties and applications", Chemistry and Industry, June 1982.

3 U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Cosmetics Fact Sheet, February 7, 1995.

4 P Hubert, O G Vitzhum, "Fluid Extraction of Hops, Spices and Tobacco with Supercritical Gases", Extraction with Supercritical Gases, Schneider, Stahl, Wilke, Verlag Chemie, Weinheim-Deerfield, Basel, 1980.

Chapter 1

Advent of a New Extraction Technique

Nearly 100 years after the first paper on supercritical fluids and associated technology was presented in 1879,[1] research in this field of interest was initiated locally.

Legislation on the so called "red scare"[2] was passed, which banned the use of synthetic red colouring in food substances, particularly in Vienna sausages. This prompted the local food industry to look for other natural sources of red colouring. Many synthetic food colours are now known to be carcinogenic.

Similar legislation in Europe, introduced by the European Economic Committee,[3] also laid down permissible levels of organic solvent and pesticide residues in extracts to be used in the food industry. This legislation forced industry to look for natural red colours.

The extraction of xantophylls from paprika soon became the focus for an alternative red pigment. Extraction techniques for capsorubin and other similar xantophylls became a top priority. The hunt for new technology was on. It had to meet the requirements of both local and international legislators. The solvent needed to be non-polluting, affordable, non-toxic and non-inflammable. The extracted oleoresin had to have no toxic residues and, in particular, no insecticides.

sc-CO_2 fitted these prerequisites remarkably well. However, suitable equipment to do this work had to be sourced and purchased. The author visited several manufacturers of high-pressure extraction equipment in the period 1980-1981 to purchase a supercritical fluid extractor destined for use on South African soil for the first time. The companies UHDE (Germany), Vereinigte Edelstahl Werke (Austria), Nova Werke (Switzerland) and Krupp (Germany) were included.

Pilot plants in operation at the University of Gratz, Austria and at the Zurich Polytechnik, Switzerland, were used to get a better understanding of the requirements for SFE equipment. An industrial plant in Berlin, capable of handling several tons of tobacco per hour, was also visited.

A suitable pilot plant scale sc-CO_2 extractor with up to 4-liter capacity was eventually ordered from Switzerland, manufactured by Nova Werke and commissioned by the author for the company CG Smith Sugar. This

instrument, after being transferred to a second company Naturex, ended up in Somerset West at Somchem and was finally donated to the supercritical fluid research group at the North-West University where the research in this dissertation could be completed after many years.

1.1 Early work

The annatto colouring from *bixin orellana*, a dye used by the "Red Indians" as war paint, was the first natural colour to be extracted in South Africa using sc-CO_2 as extractant. This took place in 1982 in the research laboratories of CG Smith Sugar on the instrument referred to above. The dye was used successfully in the dairy industry to colour cheese and butter for quite some time. It is still used in the frozen foods industry to colour "smoked" fish products.

Early efforts were very much a case of hit or miss. No suitable optimisation programs were readily available and much of the work was based on the empirical research done by Nova Werke in Switzerland.[4] The Nova Werke approach was unique. An organic extract of the sought after material, in the form of an oleoresin, was placed in a high-pressure optical cell. Carbon dioxide was let into the cell and the pressure and temperature of the cell was altered manually until the organic extract dissolved in the supercritical fluid.

This empirical approach gave the researcher an idea of the region of the phase diagram needed to be explored.[5] It had several shortcomings, the main one being that an organic solvent extract often contained no water, whereas the sc-CO_2 derived product contained up to 10% water. This amount of water was sufficient to significantly alter the extraction parameters.[6] It was shown that at ca. 400 atm and about 60^{0}C almost every extractable component was extracted.

Nova Werke produced a sc-CO_2 derived oleoresin that resembled the known organic extract most closely, insecticide and all! However, under these conditions, selectivity was virtually impossible and many other undesirable products were included in the extract. At the conditions specified above both pigment and oleoresin were extracted from annatto seed. The separating conditions were set at 60 atm and 27 ^{0}C.[7]

The red pigments extracted from *Bixin orellana* never found application in the colouring of other food products. Many factors contributed to this, one being the unavailability of a reliable supplier of the raw material, another being the failure of the attempts to cultivate *Bixin orellana* locally.

1.2 Further developments

CG Smith Research Laboratories, Merebank, Durban, under the direction of the author, were soon extracting other natural products on the newly acquired pilot plant. These included hops, onions, garlic, apples, tea, pyrethrum, turmeric and ginger.

The company Naturex[8] was formed as a subsidiary of CG Smith to expand their natural product extraction facilities. It was situated in Chamdor, near Krugersdorp. It closed its doors three years later.

Several companies throughout the world now run research programs on sc-CO_2 extraction, especially of plant material offering low-volume high-value components such as essential oils and natural waxes. One such company is the Austrian firm Natex, established in 1993. Some recognition for the early work done in South Africa may be gathered from a quote on the web page of Natex:[9]

"Sometime, somewhere in the bush of Africa, from commissioning of a conventional solvent plant, frustrated engineers started to think about an alternative technology for extraction. Today, about 20 years later, those people along with their colleagues have become experts in supercritical fluid extraction and are responsible for the excellent reputation of Natex Prozesstechnologie GmbH"

Twenty years ago, the only company in Africa involved in sc-CO_2 extraction can be proven to have been CG Smith.

1.3 Current status

Extraction using sc-CO_2 proved quite successful, but the concept has not yet been commercialised in South Africa.

Compared to conventional solvent extraction techniques for the same raw material, SFE equipment is generally more expensive with respect to capital costs, but running costs may be significantly lower in the long run, depending on the type of application.

An additional problem is that extracts are not always of the same composition as those obtained by conventional techniques. This may require marketing campaigns to convince potential buyers that the supercritical derived extract is comparable to a large extent.

References

1 J B Hannay, J Hogarth, Proc. Royal Soc. (London) 29, 324 (1879).

2 U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Cosmetics Fact Sheet February 7, 1995, Color Additives.

3 Directive 85/374/EEC.

4 H Brogle, paper presented at a meeting of the SCI Food Group Food Engineering Panel, London, February 1982.

5 H Brogle, Chemistry and Industry, June 1982, "CO_2 as a solvent: its properties and applications".

6 Brunner G, et al, "High Pressure Science Technology", 1979, 565-572, paper presented at the 6th Airapt Conference, 1977.

7 Nova Symposium on High Pressure Extraction, 1980.

8 Internal Reports, CG Smith Sugar (Pty) Ltd, 1982 and 1983, CEO Gunter Augustein, (Austrian).

9 www.natex.co.at

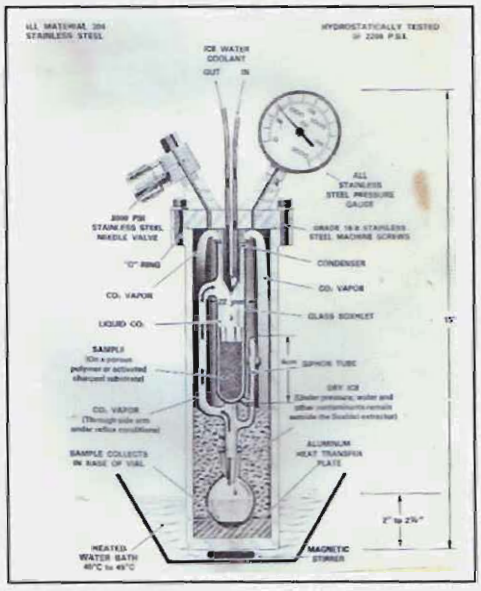
Chapter 2

Supercritical Extraction Equipment

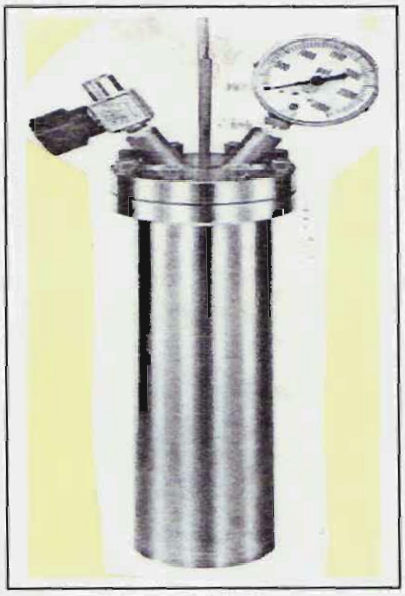
This chapter deals with various supercritical carbon dioxide extractors and pilot plants used in this study. These include a micro scale plant based on a modified HPLC, the 4-liter capacity Nova Swiss pilot plant referred to in Chapters 0 and 1, a 50-liter pilot plant used on the premises of Nova Werke, Switzerland, and a 200-liter semi-industrial plant belonging to Hopfen Extraktion Technik, Germany. A brief review of these different instruments could assist researchers in establishing the requirements that should be met by the equipment they would like to purchase.

2.1 Early sc-CO_2 equipment

The simplest way of performing sc-CO_2 extraction was with a special high-pressure vessel (Figure 2.1) in which an ordinary soxhlet apparatus was inserted.[1]



Schematic drawing of internal layout



Picture of external appearance

Figure 2.1 Early soxhlet type high-pressure carbon dioxide extractor

The extractor can be used with any substance which can be liquefied at temperatures between 0°C and 20°C and a pressure of 100 atm. Since the entire assembly is contained inside the extraction vessel, ordinary glassware can be used. The sample material is placed in a soxhlet thimble as usual. Carbon dioxide is most easily supplied in the form of dry ice. As the dry ice vaporises in the closed assembly, pressure rises to between 60 and 100 atm. Ice water is passed through the cold finger condenser. When condensation begins, the pressure drops to about 40 to 50 atm. At these pressures, any water or other impurities introduced with the dry ice will remain outside the extraction assembly, while pure carbon dioxide condenses back into the soxhlet. Several hours may be required to complete an extraction. Once the extraction has been completed, the apparatus is placed in a shallow pan of dry ice and the safety valve is opened to release any pressure before the unit is opened.

2.2 Extractors employed in this study

The work in this investigation was performed with the four different extractors listed in the introductory paragraph of this chapter. The technical details of these four units are given in the paragraphs below.

2.2.1 Micro scale extractor[2]

A suitable micro scale SFE unit was built by modifying an HPLC to utilise liquid CO_2 as solvent.[3] This required a heat exchanger machined to fit the pump head and a cooling bath capable of maintaining temperatures near 0 °C. A 6 mm internal diameter stainless steel chromatographic column made an excellent extraction "chamber" having a volume of between 10 and 20 mL. The outlet from the column passed through a restrictor in order to have a more constant pressure within the extraction "chamber". A schematic diagram of the extractor is given in Figure 2.2.

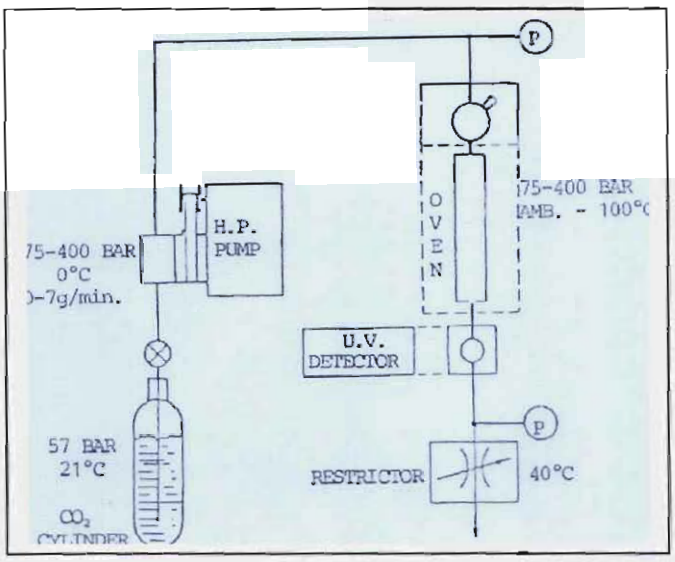


Figure 2.2 Schematic diagram of a modified HPLC for SFE

By analysing the raw material before and after an experimental run, a measure of the efficiency of extraction can be obtained for any chosen condition. The advantages of using the modified HPLC included accurate measurement of flow, temperature and pressure by utilising its superior sensing devices and instrumentation. The micro scale work enabled one to obtain basic operation parameters for larger plants in a relatively short time.

2.2.2 Nova Swiss 4-liter pilot plant

The Nova Swiss 4-liter supercritical pilot plant is capable of extracting batches of up to 1 kg of material. The instrument is shown in Figure 2.3. A major problem is the inaccuracy of the instrumentation. Pressures vary by ca. 25 atm on either side of the set parameter, the temperatures of the extraction and separation vessels are difficult to maintain, and the flow is limited to what can be produced by two membrane compressors.

A worthwhile modification was to insulate the extraction and separation vessels in order to obtain more accurate temperature readings. The extraction time largely depends on the flow rate or the amount of CO_2 circulated through the extractor. A CO_2 circulating pump was introduced as a further modification of the original instrument to operate either on its own or in conjunction with the existing compressors. The fluctuations in pressure largely resulted from the use of pneumatic instead of electronic pressure sensors and controllers.

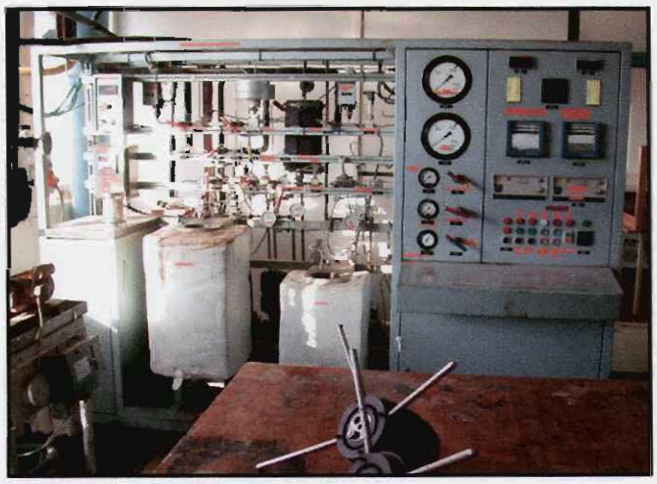


Figure 2.3 Nova Swiss 4-liter extraction plant currently used at North-West University, Potchefstroom Campus

In order to speed up the handling of material before and after extraction, a close fitting insert for loading into the extractor was designed. Once material has been extracted, the insert is removed and another insert filled with new material is placed into the extractor.

2.2.3 Nova Werke 50-liter plant

Parameters established on the 4-liter plant needed to be confirmed on a larger plant so that calculations for scaling up of the extraction process could be verified. This was done on a 50-liter pilot plant in Switzerland owned by Nova Werke and situated at Embatiken, near Maur.

This plant has all the inherent drawbacks (e.g. unstable operation as a result of pneumatic instead of electronic control) of the 4-liter plant discussed above, but it was modified in several ways.

A multi-level thermocouple was installed in the separator to indicate the level of CO_2 liquid. This was later implemented on the 4-liter plant. The separator was also fitted with a spiral heating tube connected inside the separator to improve temperature control.

A double-headed pump was used instead of the compressors of the 4-liter plant. The problems associated with diaphragm failure were thus eliminated. However, this pump was largely under-sized in terms of carbon dioxide delivery for efficient extraction. It was capable of a pressure of maximally 350 atm and a flow rate of only 50% of that of the 4-liter pilot plant.

The plant had two extractors and two separators and used a "carousel" technique in which partially spent material is extracted with fresh CO_2 and fresh material with "used" CO_2. The developers of this technique claimed that they were able to extract more of any desired material within a given time.

One of the major differences between the 4-liter pilot plant and this larger unit is the provision for CO_2 storage and recovery. The former only requires a cylinder, whereas the latter has a CO_2 storage tank installed on a weighbridge and maintained at -20 ^{0}C and 20 atm using a suitable chiller unit.

2.2.4 Hopfen Extraktion Technik 200-liter plant

Many of the inadequacies of the smaller 4-liter and 50-liter pilot plants were overcome by employing a 200-liter semi-industrial plant of Hopfen Extraktion Technik at Wolznach in Germany, a typical view of which is shown in Figure 2.4. The purpose of work carried out on this plant was to verify results obtained with the 4-liter and 50-liter plants above with a view to scaling up some laboratory work.

The plant consists of a 200-liter extractor and three 20-liter separators. Carbon dioxide is circulated by a pump from an on-line surge tank stored at room temperature and at 60 atm. It has a capacity of more than 1 m^3 and can store carbon dioxide vented from the extractor after each run to avoid loss of extract left behind in the fluid.

Figure 2.4 Typical 200-liter plant

The extraction vessel, manufactured by the well-known company UHDE is fitted with a tight-fitting lid, sealed with an o-ring and held in place by two semi-circular clamps fitting around the vessel. It has no insert and thus 80% of its theoretical volume is available for sample material. Three 20-liter separators operate in series as separation is incomplete after only one separator because of the high flow rate of the sc-CO_2. The instrumentation on this plant is of a high standard, and microprocessor control made it easy to maintain the desired temperatures and pressures.

2.3 Current status of extraction equipment

Figure 2.5 Research plant of Krupp Research Institute for high-pressure extraction

Table 2.1 Recently commissioned supercritical fluid extraction plants
(before 1992 executed under SCHOELLER-BLECKMANN)

YEAR	SUPPLY	PLANT SIZE	COUNTRY
1983	Complete Multi Purpose Plant	30 l, 300 bar, 100°C	Austria
1985	**Multi purpose Pilot Plant**	**3 x 35 l, 300 bar, 100°C**	**Austria**
1986	2 High Pressure Autoclaves, Finger -Pin -Closure	700 mm, 100 bar, 300°C	Germany
1987	Turn Key Plant, Food Industry 3 High Pressure Autoclaves, Finger -Pin -Closure Extractor Baskets, Food Industry	35 m³ total, 325 bar 700 mm, 100 bar, 300°C 3 x 200 l	Germany Germany Germany
1988	Liquid / Liquid Column, Food Industry 2 High Pressure Autoclaves, Finger -Pin -Closure 1 High Pressure Autoclaves, Finger -Pin -Closure Extractor Baskets, Food Industry	325 bar, 120°C 600 mm, 150 bar, 300°C 800 mm, 125 bar, 250°C 15 x 6500 l, 15 x 1500 l	Germany Germany Germany Germany
1989	Turn Key Plant, Food Industry 1 High Pressure Autoclaves, Finger -Pin -Closure	approx 63 m³ total 800 mm, 125 bar, 250°C	Italy Germany
1990	Extension of a Multi Purpose HPE Pilot Plant (Extractor), Food Industry Liquid / Liquid Column, Food Industry	100 l, 550 bar DN 45, 325 bar	Austria Austria
1991	Additional Order for the Turn Key Plant		Italy
1993	Complete Multi Purpose Pilot Plant Complete Multi Purpose Plant Dealcoholization Pilot Plant	5 l, 1000 bar 200 l, 550 bar 100 l/h	Austria Czech Austria
1985 till 1994	Serveral Confidential Investigation Orders for Process / Product Optimization for Food - and Chemical industries		Europe USA Far East
1994	Lab Scale Unit	5 l, 550 bar	Hungary
1995	Turn Key Multi Purpose Plant Turn Key Multi Purpose Plant Lab Scale Unit	2 x 800 l, 550 bar 2 x 250 l, 550 bar 5 l, 1000 bar	India India India
1995	Product / Process Development - Food Industry		Europe
1996	Turn Key Multi Purpose Plant Lab Scale Unit	3 x 300 l, 550 bar 5 l, 1000 bar	India India
1997	Product - Development - Pharma Industry Depestisation Plant	3 x 5800 l, 325 bar	Europe Far East
1998	Lab Scale Unit	5 l, 1000 bar	Germany

References

1 J & W High-Pressure apparatus, J & W Catalogues.

2 D MeManigill, R Board, D Gere, "Hardware Adaptations to HPLC Apparatus to Enable Operation as a Supercritical Fluid Chromatograph", Hewlett-Packard Company, Avondale, Pensylvania 19311.

3 R Gere, Hewlett-Packard Research Laboratories, Pennsylvania, personal communication, 1982.

Chapter 3

Experimental Operations

Several natural products were selected and extracts obtained from these by sc-CO_2 in fulfilment of the objectives of this study. These included annatto, pepper and coffee. The origin of the plant material, sample preparation, chemical analysis, and data acquisition and processing are the experimental operations dealt with in this chapter.

3.1 Materials and reagents

Analytical Grade (AR) reagents were used throughout the study. Unless stated otherwise, chemicals were supplied by Merck.

- **Annatto**

Seeds of the plant *bixin orellana* imported from South America were used. Attempts to cultivate this plant in northern Kwazulu-Natal have not met with success. Analytical reagents included acetone and dichloromethane.

- **Pepper**

Ground black pepper corn was purchased from a local supermarket. Pepper oleoresin was prepared by soxhlet extraction of ground pepper corn using dichloromethane. Other reagents included ethanol (96%), acetonitrile, acetic acid (1%), pentane, dichloromethane and piperine (purum grade, 98%) and hexacosane (C_{26}, 99%) from Fluka.

- **Coffee**

Washed and dried raw coffee beans were obtained from TW Beckitt. Determination of caffeine content required the following analytical reagents: 10% (m/v) lead acetate solution, chloroform, 5% (m/v) sodium hydroxide, anhydrous sodium sulphate and ethanol (96%).

3.2 Extraction

The selected plant materials were prepared for extraction with both organic solvents and sc-CO_2 by grinding the dried material to a fine mesh to ensure proper penetration by the solvent/fluid. Preweighed samples of the different types of material were subjected to either solvent extraction by shaking the portion in a suitable solvent for a given period of time, followed by soxhlet extraction techniques, or to sc-CO_2 extraction by using one or more of the supercritical extractors described in the previous

chapter and selecting a specific temperature and pressure (or density), flow rate, duration and amount of added organic cosolvent for each run.

3.3 Analysis[1-5]

For all sample analysis, a combination of spectrophotometric (UV/VIS) and chromatographic (GC and/or HPLC) techniques proved to be adequate. Instruments mostly used were a Hewlett-Packard 8450 spectrophotometer and a Hewlett-Packard 8890 gas chromatograph, unless stated otherwise.

- **Annatto**

HPLC analysis of annatto extract was performed by dissolving and diluting 20 times (5 mL → 100 mL) 0.1 g of extract or oleoresin in dichloromethane.

UV/VIS analysis of annatto colouring was done by dissolving and diluting 20 times (5 mL → 100 mL) 0.1 g of extract or oleoresin in acetone. A suitable amount of the solution prepared above was transferred to a standard cuvette and a UV/VIS absorption spectrum was obtained. The % total pigment was calculated according to the ASTA[2] method. No distinction was made between yellow or red pigments and extracts were simply analysed by quantitative comparison of the respective absorption spectra.

- **Pepper**

Quantitative analysis of pungent principals of pepper oleoresins (piperine and its derivatives) were carried out using UV/VIS, HPLC or GC. It seemed that HPLC and GC results, considering only the peak of piperine, were comparable. In the same way, HPLC and UV/VIS gave similar results for the total amount of alkaloids.

0.10 g oleoresin was dissolved in 100 mL ethanol (96%) and this solution was diluted 125-fold for UV/VIS and 5-fold for HPLC analysis using ethanol.

Absorbances were measured in a 1 cm silica cell at 343 nm against the pure solvent as reference. Piperine was quantified by an external standard method. Standard solutions were prepared by dissolving pure piperine in ethanol in concentrations of 0.005 to 0.00125 g/L. Other alkaloids were quantified by comparing them to piperine, assuming the same response factors as those for piperine.

The HPLC conditions for the analysis of pepper were as follows:

Column:	Ready-packed column (250 x 4.6 mm) of 5 μm Nucleosil 120 A (a fully-capped C18 bonded phase).
Mobile phase:	48% (v) acetonitrile - 52% (v) water (1% acetic acid).
Flow rate:	1 mL/min
Injection volume:	40 μL
Detector UV:	254–280–343 and 364 nm.
Run time:	45 min to allow elution of all the minor UV absorbers
Piperine RT:	20 min

The GC analysis of pepper was performed on a capillary polar column BP1 (50 m x 0.22 mm I.D., 0.25 μm film thickness) connected to an FID detector. The operating parameters: hydrogen as carrier gas at 1 mL/min, split at 25 mL/min, injector temperature at 300°C, detector temperature at 300°C. The program conditions were 250°C to 280°C at 0.5°C/min. (Run time was ca. 35 min, piperine retention time ca. 20 min and hexacosane retention time ca. 13 min.) Alkaloids were quantified by using hexacosane as internal standard. The standard solution was prepared by adding 2 mL of pure piperine solution (4 g/L in dichloromethane) to 1 mL of hexacosane solution (3 g/L in pentane).

- **Coffee**

HPLC and UV/VIS analysis of caffeine required raw coffee beans to be ground to pass through a 600 μm (60 mesh) sieve.

The spectrophotometric analysis of the caffeine content of ground coffee beans entailed the following: Approximately 1 g of the finely ground material was weighed accurately to 4 decimal places and transferred into a 500 mL round bottom flask and refluxed for 2 hours in ca. 100 mL distilled water. The cooled solution was quantitatively transferred to a 250 mL volumetric flask and diluted to the mark. 50 mL of the supernatant liquid was pipetted into a 200 mL beaker to which 6 mL of 0.2 % lead acetate solution was added. The mixture was boiled for 5 minutes to precipitate organic acids. The cooled solution was transferred quantitatively to a 100 mL volumetric flask and made up to the mark with distilled water. From the settled solution, a 50 mL aliquot was pipetted from the supernatant liquid for extraction. This portion was extracted with 5x 5 mL portions of AR grade chloroform in a separating funnel. The combined extracts were washed with 4 mL of a 0.1% sodium hydroxide solution. These were then washed with a further 10 mL distilled water. The washed solution was transfered to a 50 mL volumetric flask and made up to the mark with chloroform. The absorbance at either 272 nm or 276 nm was measured. The mass of caffeine was read off from a suitably prepared calibration graph. The % caffeine was then calculated as follows:

$$\% \text{ caffeine} = \frac{(\mu g \text{ caffeine from the graph})(250)(100 \times 10^{-4})(50)}{(g \text{ sample taken})(50)(50)}$$

$$= \frac{(\mu g \text{ caffeine from the graph})(2000)}{g \text{ sample taken}}$$

3.4 Data processing software

All extraction experiments conducted were statistically analysed using multiple linear regression techniques[6]. The computer program written in Basic by the author is included at the end of this chapter. It was written in such a way that by changing only one statement, the curve fitting part of the program could be changed to either logarithmic, exponential or geometric regression techniques.[6] In all cases, the best fit for the experimental curve was found to be a linear regression one.

Individual experiments were chosen in such a way as to have the largest possible variation of parameters in order to ensure that the numerical mathematics of the program converges to a single solution.[7]

These regression techniques are also available as standard programs in Microsoft Office, but they do not allow the user to change the regression analysis from being a multiple linear regression analysis to any other suitable curve fitting technique. However, optimisation of the desired result was done using standard regression and linear programming techniques, which are available on Microsoft Excel Office 2000.

In all cases, the statistical coefficient of correlation was expected to range from 0.95 to 1, with 1 being the indication of an ideal or "perfect" statistical fit.

BASIC Program

```
5 REM THIS PROGRAM IS CALLED PAPCOMP ..ALL EXTRACTION WORK
10 REDIM X(20), DD(20), E(20), A(20, 20), V$(120)
12 REDIM H(20, 20)
15 DIM M, N, Z$
20 READ M, N
30 CLS
40 LPRINT "                              MULTIPLE LINEAR REGRESSION"
45 LPRINT "": LPRINT ""
50 LPRINT "# OF VARIABLES =", M
60 LPRINT "# OF EQUATIONS =", N
61 LPRINT ""
62 LPRINT ""
65 LPRINT "  "
66 LPRINT "                * * *       I n v e s t I g a t I o n      * *
*"
70 FOR I = 1 TO M + 1
80 READ V$(I)
87 Z$ = "EQUATION"
90 NEXT I
92 LPRINT ""
95
110 FOR I = 1 TO M + 2
120 FOR J = 1 TO M + 1
130 A(J, I) = 0
140 NEXT J
150 DD(I) = 0
160 NEXT I
161 FOR K = 1 TO N
162 FOR I = 1 TO M + 1
163 READ X(I)
164 H(K, I) = X(I)
165 NEXT I
167
168 NEXT K
169 LPRINT ""
170 FOR K = 1 TO N
190 FOR I = 1 TO M + 1
200 X(I) = H(K, I)
210 NEXT I
220 DD(M + 2) = DD(M + 2) + X(M + 1) ^ 2
230 A(1, M + 2) = A(1, M + 2) + X(M + 1)
235 DD(1) = A(1, M + 2)
240 FOR I = 1 TO M
250 A(1, I + 1) = A(1, I + 1) + X(I)
255 A(I + 1, 1) = A(1, I + 1)
260 A(I + 1, M + 2) = A(I + 1, M + 2) + X(I) * X(M + 1)
265 DD(I + 1) = A(I + 1, M + 2)
270 FOR J = I TO M
280 A(J + 1, I + 1) = A(I + 1, J + 1) + X(I) * X(J)
285 A(I + 1, J + 1) = A(J + 1, I + 1)
290 NEXT J
300 NEXT I
310 NEXT K

360 A(1, 1) = N
370 FOR I = 2 TO M + 1
380 E(I) = A(1, I)
390 NEXT I
400 FOR S = 1 TO M + 1
```

```
410 FOR T = S TO M + 1
420 IF A(T, S) <> 0 THEN 460
430 NEXT T
440 LPRINT "NO UNIQUE SOLUTION"
450 GOTO 1180
460 GOSUB 560
470 C = 1 / A(S, S)
480 GOSUB 620
490 FOR T = 1 TO M + 1
500 IF T = S THEN 530
510 C = -A(T, S)
520 GOSUB 660
530 NEXT T
540 NEXT S
550 GOTO 700
560 FOR J = 1 TO M + 2
570 B = A(S, J)
580 A(S, J) = A(T, J)
590 A(T, J) = B
600 NEXT J
610 RETURN
620 FOR J = 1 TO M + 2
630 A(S, J) = C * A(S, J)
640 NEXT J
650 RETURN
660 FOR J = 1 TO M + 2
670 A(T, J) = A(T, J) + C * A(S, J)
680 NEXT J
690 RETURN
695
696 LPRINT "  "
700 LPRINT "PREDICTION EQUATION:"
710 LPRINT V$(M + 1); " = "
720 LPRINT A(1, M + 2); " +"
730 FOR T = 2 TO M
740 LPRINT A(T, M + 2); "*"; V$(T - 1); "+"
750 NEXT T
760 LPRINT A(M + 1, M + 2); "*"; V$(T - 1)
770 LPRINT "  "
780 S = 0
790 FOR I = 2 TO M + 1
800 S = S + A(I, M + 2) * (DD(I) - E(I) * DD(1) / N)
810 NEXT I
820 T = DD(M + 2) - DD(1) ^ 2 / N
830 C = T - S
840 I = N - M - 1
850 J = S / M
855 K = 0
860 K = C / I
870
880 LPRINT "  ", "REGRESSION TABLE"
890 LPRINT "  "
900 LPRINT "SOURCE", "SUM OF SQ", "DEG. FREEDOM", "MEAN SQ"
910 LPRINT "REGRESSION", S, M, J
920 LPRINT "RESIDUAL", C, I, K
930 LPRINT "TOTAL", T, N - 1
940 LPRINT "  "
970 LPRINT "F="; J / K
980 LPRINT "  "
990 J = S / T
1000 LPRINT "COEFF. OF DETERMINATION= "; J
```

```
1010 LPRINT "COEFF. OF MULTIPLE COERRELATION = ", SQR(J)
1020 IF C / I < 0 THEN 1050
1030 LPRINT "STANDARD ERROR OF ESTIMATE = "; SQR(C / I)
1040
1050
1055
1060
1070
1075 FOR J = 1 TO N
1080 S = A(1, M + 2)
1090
1095 FOR I = 1 TO M
1110
1120 X(I) = H(J, I)
1130 S = S + A(I + 1, M + 2) * X(I)
1140 NEXT I
1150 H(J, M + 3) = S
1155 NEXT J
1160
1170
1180 LPRINT "": LPRINT ""
1185
1190 REM DATA STATEMENTS MUST BE:
1200 REM D1:# OF VRBLS,# OF EQNS
1210 DATA 8,11
1220 REM D2:"VRBL NAMES","NAME OF Y"(MAX 6 CHRS)
1230 DATA
"ASTA","WATER","BATCH","PRESS","TIME","TEMP","FLOW","KG/KGPAP","%YIELD"
1240 REM DATA STATEMENTS WITH COEFTS. OF VARIOUS EQNS.
1250 DATA 130,4.18,1200,290,1.63,24,2.56,3.62,34.2
1260 DATA 130,3.63,600,440,.97,24,1.61,7.7,14.9
1270 DATA 138,3.62,1200,200,4.8,21,2.92,12.12,46.7
1280 DATA 140,3.25,600,100,2.98,22,1.02,5.23,22.1
1290 DATA 129,1.84,600,190,1.23,21,2.99,6.25,7.1
1300 DATA 46,7.26,600,200,7.11,21,2.92,37.27,28
1310 DATA 141,7.99,700,200,10,21,2.92,45.34,34
1320 DATA 132,7.04,500,300,5,60,2.48,26.67,39
1330 DATA 213,5.57,1012,500,5.4,93,7.3,41.26,81.45
1340 DATA 52.6,5.57,425,563,2.6,96,.0,3.24,56.84
1350 DATA 186,5.6,430,491,2.2,97,0.55,2.98,15.6
1351 LPRINT "         O   R  I  G  I  N  A  L        D  A  T  A
EXP//THEORY"
1352  LPRINT "   "; V$(1); "    "; V$(2); "   "; V$(3); "   "; V$(4); "
"; V$(5); "    "; V$(6); "   "; V$(7); "   "; V$(8); "      "; V$(9)
1354  LPRINT "   "; "VALUE"; "      "; "%"; "    "; "GRAMS"; "   ";
"BARS"; "     "; "HOURS"; "     "; "oC"; "   "; "kgCO2/Hr"
1356  FOR K = 1 TO N
1357  LPRINT USING "#####.##"; H(K, 1); H(K, 2); H(K, 3); H(K, 4); H(K,
5); H(K, 6); H(K, 7); H(K, 8); H(K, 9); H(K, M + 3)
1358  NEXT K
1359
```

References

1 Isolation and identification of new apocarotenoids from annato (*Bixa orellano*) seeds. Analytical Abstracts Apr. 1997.

2 Standard Methods of the American Spice Traders' Association.

3 High-performance liquid-chromatographic separation and spectral characterisation of the pigments in turmeric and annato. Analytical Abstracts Dec. 1988.

4 A.Z. Mercadante, A. Steck & H. Pfander, Three minor carotenoids from annato (*Bixa orellana*) seeds. Phytochemistry 1999, 52, 135-139.

5 Detection of annato dyestuffs, norbixin and bixin in cheese by means of derivative spectroscopy & high-performance liquid chromatography. Analytical Abstracts Apr. 1988.

6 Analysis, 1999, 27, 69-74 EDP Sciences, Wiley-VCH.

7 Numerical Methods with Fortran IV Case Studies, WS Dorn, et. al, Wiley, 1972.

Chapter 4

Theoretical Aspects

Much has been published about the theory of supercritical fluid extraction (SFE), but many aspects still need to be considered by a researcher before commencing SFE on new material. In this chapter mainly those theoretical aspects which have practical importance, such as to select process parameters and to optimise these for extraction, are considered. Specifically, answers should be found as to

- how firmly should the extractor be packed, i.e. is any significant channeling of CO_2 possible?
- what are the optimum conditions while maintaining cost at a minimum?
- what is the solubility of the target substance in CO_2?
- what flow rate, pressure and temperature should be used?
- what effect will a polar or non-polar cosolvent have?

In this investigation a simple approach was used throughout to answer these questions.

4.1 A multivariant approach

Attempts to optimise extraction conditions are mostly based on an approach by virtue of which an extract is obtained by using sc-CO_2 at selected temperatures and pressures and then analysing for a desired ingredient. All other ingredients in the extract are totally ignored. The conditions which produce the highest amount of the target compound are then chosen, irrespective of the nature of the composition of the extract or the cost involved.

At first glance, the graph in Figure 4.1 suggests that the optimum condition for the extraction of piperine in pepper is about 300 atm at the selected temperature of 60 ^{0}C. However, at this pressure other unwanted material is also produced, as was shown by the author.[1]

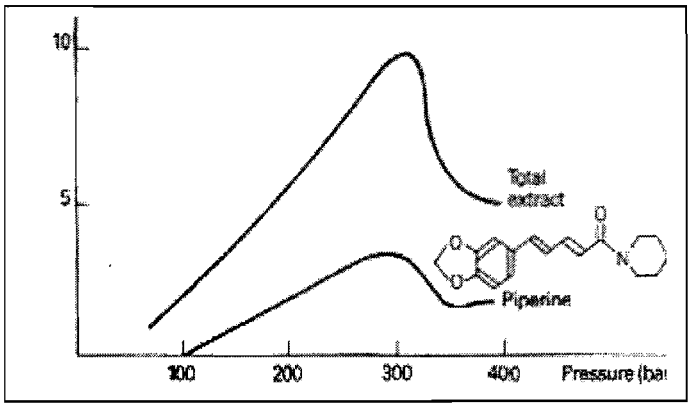


Figure 4.1 CO_2 extraction of piperine from pepper at 60 ^{0}C for 3 hours. The vertical axis represents the % extracted by mass.

Important parameters such as raw material analysis, CO_2 flow rate, bulk density of the sample, optimum time of extraction for maximum yield, solubility in CO_2 at different conditions, to mention but a few, are all ignored.

One may obtain the best conditions (350 atm, 60 ^{0}C) for extraction from results such as those in Figure 4.1, but the extraction efficiency is not reflected. This is typical of work done by many researchers who made similar empirical deductions without taking all parameters limiting the extraction into consideration.

The piperine content of ground black pepper determined in this study is 6.5%. The maximum piperine extracted according to Figure 4.1 is 3.2 % only[1], which is less than half the extractable content. The difference is attributed to using a multivariant approach in this study, which relates the raw material used and the conditions of extraction to the actual yield obtained. It will be discussed in detail in Chapter 6, which deals with the extraction of piperine from pepper.

The fact that any substance which dissolves in sc-CO_2 alters the solvation properties and even the critical constants of the fluid, is often ignored. Each foreign substance in the CO_2 acts as an additional entrainer, with either hydrophobic or hydrophilic effects. Water content, as will be shown later, has an effect on the extraction of piperine, probably as a result of the hydrophilic nature and the increase in polarity of the sc-CO_2. Light oils, to the contrary, cause a hydrophobic effect and appear to have a negative effect on the extraction of piperine. Usually, a combination of these effects is found and, as can be seen from work done in this investigation (Chapters 5-7), water and light oils are determining factors in the extraction processes studied. The effect of increasing or decreasing the polarity of CO_2 relates to changing its hydrophilic or hydrophobic nature by virtue of a cosolvent or modifier.

Once an equation is established which describes the relationship between the yield of extract and the different parameters contributing to it, it is possible by applying standard techniques and suitable computer programs to answer the question "what if". Such programs are readily available in professional packages[2] capable of optimising a result by manipulating those parameters contributing to the final result. It is also possible to investigate mathematically the effect of one such parameter while all other parameters are kept at a constant value. In later chapters of this dissertation, a comparison between the actual yield of a desired ingredient at certain conditions and the theoretical predictions by regression techniques is made. Generally these agreed to within 2 %.

4.2 Empirical deductions

Early attempts to predict the kind of extract that could be obtained for a given set of extraction parameters were formulated empirically by researchers at Nova Werke. Their approach was unique in that they started with conventional oleoresin extract or an essential oil and proceeded to find the extraction conditions which would dissolve the material. This was done by using their specially designed high-pressure optical cell and adjusting the conditions of pressure and temperature until the oleoresin or essential oil was completely

dissolved in the sc-CO_2.[3] A summary of their results is given in Table 4.1. This generally holds true for the pure products free of water. In general, it can be said that as the pressure is increased at a constant temperature, more and more of the pure compounds become soluble in the sc-CO_2, in the order specified, depending on the nature and type of raw material being extracted.

Table 4.1 Results obtained by Nova researchers

Pressure range (atm)	**Temperature range (°C)**	**Product obtained**
50-70	30-80	Deodorisation
70-110	30-80	Essential oils
110-170	30-80	Free fatty acids
170-220	30-80	Oils
220-270	15-80	Total extract (pale)
280-350	10-80	Total extract

The more volatile essential oils were extracted first (73 atm), then higher terpenes and esters, followed by free fatty acids, fatty oils, waxes, resins, and finally pigments, i.e. carotenoids (350 atm). Combining the results in Figure 4.1 with those of the Nova researchers, a more complete Table 4.2 can be compiled.

Table 4.2 Combination of results obtained by Nova researchers and authors of Figure 4.1

Pressure range (atm)	**Temperature range °C**	**Product obtained**
20-50	20-30	Water
50-70	30-80	Volatile essential oils
70-110	30-80	Pepper essential oils
110-170	30-80	Free fatty acids
170-220	30-80	Common vegetable oils
220-270	15-80	Total extract (pale)
280-350	10-80	Total extract (dark)

In some cases it is desirable to have extraction conditions at which CO_2 is practically "dry". This would be when non-polar properties of the gas are preferred, e.g. for extraction of fragrances from fruit or vegetables (apples, grapes, tomatoes) which contain relatively large amounts of water. In other instances a more polar CO_2 extraction is required and water plays an important role, e.g. substances like caffeine and nicotine.

4.3 Trends in alkaloid extraction

Attempts by other researchers to establish conditions for the extraction of pure alkaloids with either pure CO_2 or pure NO_2 from glass wool at 40°C gave no clear indication of any trend. Comparisons were made between functional groups,

melting points, boiling points, number of carbon atoms and even mole masses, but to no avail.[4] Their comments are worth noting:

(a) Hydrocarbons and other typically lipophilic organic compounds of relatively low polarity, i.e. esters, ethers, lactones and epoxides, can be extracted in the lower pressure range, i.e. 70-100 atm.

(b) Introduction of strongly polar functional groups (e.g. -OH, -COOH) makes extraction more difficult. In the case of benzene derivatives, substances with three phenolic hydroxyls are still capable of being extracted, as are those benzene derivatives with one carboxyl and two hydroxyl groups. Substances in this range which cannot be extracted are those with one carboxyl and three or more hydroxyl groups.

(c) More strongly polar substances, such as sugars and amino acids, cannot be extracted in the range up to 400 atm without a cosolvent or entrainer.

(d) Fractionation occurs in the pressure gradient where there are large differences between conditions for boiling or sublimation, i.e. differences in volatility and/or marked differences in the polarity of the substances. The fractionation effects are most marked in the range where there is a sharp rise in the density and dielectric constant of liquid carbon dioxide.

4.4 Polarity

An over-simplification often used to explain why certain solvents dissolve certain solutes is the phrase "like dissolves like". Solvents usually dissolve substances which have similar polarities to those of the dissolving solvent. Carbon dioxide undergoes a shift in polarity with a change in pressure, which is at a minimum at its critical pressure of 73.04 atm. Although a limited amount of water can dissolve in CO_2 at different temperatures, these amounts together with the natural changes in polarity are significant enough to influence the solubility of compounds in CO_2 as discussed earlier in this chapter.

In order to successfully employ CO_2 as an extracting agent, polarity must be controlled, or even altered, to improve its selectivity for specific compounds. The property of CO_2 to undergo changes in polarity with changes in temperature and pressure makes it such a good solvent. A common technique to adjust polarity is to introduce solvents of different polarity (entrainers) with the sc-CO_2 during extraction. The right choice of entrainer enhances the capacity of CO_2 to either dissolve non-polar or polar components from the raw material.

4.5 HETP

Calculation of the height equivalent of a theoretical plate for SFE is based on the same concepts used in chromatographical[5] separation work. In chromatography, the column efficiency is defined as

$$CE = \frac{16Y^2}{X} \text{ x theoretical plates}$$

where Y = time from the injection of a tracer sample to the center of the separation peak on the chromatogram for the substance under consideration

X = time taken for all of the substance to be eluted, i.e. the width at the base of the peak on the chromatogram. CE is a function of the column length.

In order to compare the efficiencies of different columns, we can calculate the height equivalent of a theoretical plate as

$$\text{HETP} = \frac{length}{efficiency}$$

where the column length refers to the actual height of the packed portion of the extractor. In general, the smaller the value of HETP, the more efficient the column is likely to be.

In SFE work it is difficult to obtain these values without a suitable detector linked to the plant. However, a reasonable estimate can be obtained if Y is taken as half the time to extract 100% of the extractable portion of a particular group of compounds and X is taken as the total time to extract 100 % of the sought after group of compounds in an extraction. From these values of column efficiency, the effect of flow rates, particle size of raw material, bulk density and time for a particular extraction can be estimated by conducting the necessary chemical analysis on the extracts obtained.

The term "column efficiency" should not be confused with the term "extraction efficiency". The former merely relates to the physical nature in which the material to be extracted is packed in a column. It is an empirical relationship and serves to indicate which combination of the physical properties will bring about a more efficient separation column. This is an indication of the best physical conditions, i.e. bulk density and particle size in order to separate a particular group of substances. The term "extraction efficiency" describes how much of a particular sought after group of substances has been extracted under the preset extraction condition with time.

When working with larger supercritical fluid extraction plants, these criteria become quite significant. A column which is too tightly packed, i.e. in which the bulk density is too high, or where the particle size of the ground material is too fine, can soon become completely blocked. Channeling of CO_2 can also cause problems.

A prior knowledge of the HETP of a particular extractor, e.g. for powders, could foresee the problems caused by transferring material into the separator where

blockages in the system may occur. As an illustration of the foregoing, the HETP for the 4-litre pilot plant is calculated below. It is based on experiments to establish the time required to extract the maximum possible amount of desired paprika oleoresin from paprika powders, using a pressure of 350 atm, a temperature of 56 ^{0}C and a flow rate of 2 kg CO_2 per hour. The results are tabulated below.

TABLE 4.3: % Efficiency versus time for sc-CO_2 extraction of paprika oleoresin from paprika powder

% extraction efficiency	**kg CO_2 used per kg paprika**	**time (h)**
10 24	10	5.0
20 32	13	6.5
30 38	15	7.5
40 43	17	8.5
50	19	9.5
60	23	11.5
70	28	14.0
80	39	19.5
90	56	28.0
98	100	50.0

The column efficiency can be calculated as

$$CE = \frac{\text{(half the time taken to extract 100\% of the extractable material)}}{\text{total time required for extraction}}$$

$$= (9.5)^2/45.10$$

$$= 2.01\ ^6$$

Note: The total time required for the extraction is 50 h, but the time taken to establish equilibrium is 4.9 h (from x-intercept of kinetics curve), hence the denominator equals 50 - 4.9 = 45.1 h.

$$HETP = \frac{\text{column height or length}}{\text{column efficiency}}$$

$$= 0.30/2.01$$

$$= 0.15$$

In order to reproduce the same physical column/extractor properties on a larger plant, the HETP should be as close as possible to 0.15.

The graph of % efficiency versus time in Figure 4.2 reflects the kinetics of the situation and, interestingly enough, does not pass through the origin, an indication that establishing equilibrium for the extraction (dissolution) process is not established instantaneously. Several researchers have observed that each substance dissolved in sc-CO_2 alters its critical point and nature as solvent.[7]

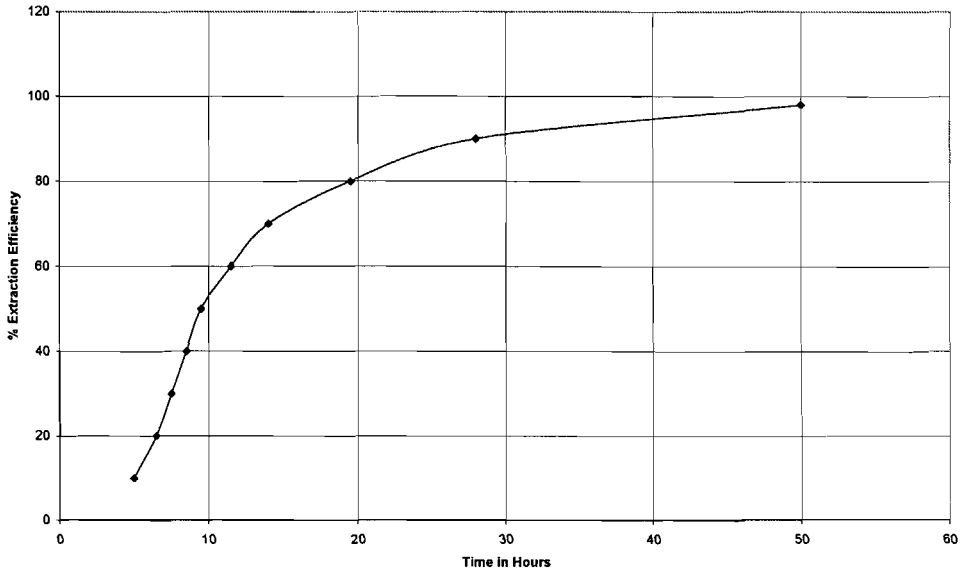


Figure 4.2: Extraction efficiency versus time

4.6 Fractional extraction and separation

By adjusting the pressure throughout an extraction run at different times, it is possible to selectively "fractionate" the various types of compounds from each other. This is sometimes referred to as "fractional extraction." In fractional separation extreme extraction conditions are chosen, e.g. 350-450 atm pressure and 60-80 ^{0}C. Several separators are run in series, set at varying pressures and temperatures, to selectively separate various types of compounds. This method is not very popular. At subcritical temperatures and pressures water is easily removed from natural products and the effect of water on the polarity of sc-CO_2 is thus easily minimised. Typical conditions for "de-watering" of a natural product, without extracting any other components, would be at pressures between 20 and 50 atm and at temperatures between 25 ^{0}C and 30 ^{0}C.

4.7 Nature of bonding in CO_2

Studies on the effect of various entrainers or modifiers leads one to wonder what role they really play. Although polarity is adjusted, there may be more contributing factors. One requires, for instance, more water as an entrainer than ethanol to achieve the same result using the same conditions, despite of the fact that water is more polar! The true cause for solubility in CO_2 is indeed complex.

References

1 BTM Clayton. Analysis conducted on pepper.

2 Microsoft Office Professional 2000, Data analysis, Excel.

3 H Brogle, Chemistry and Industry, June 1982.

4 E Stahl, W Schilz, E Schuetz and E Willing, A Quick Method for the MicroAnalytical Evaluation of the Dissolving Power of Supercritical Gases, Symposium "Extraction with Supercritical Gases", Essen, June, 1978.

5 Instrumental Methods of Chemical Analysis, Ewing, McGraw-Hill, 1975.

6 Skoog and West, Analytical Chemistry, John Wiley & Sons, 1977.

7 Personal communication, Dr. Dennis Gere, Hewlett-Packard Research Laboratories, Avondale, Pensylvania.

Chapter 5

sc-CO_2 Extraction of Annatto (*Bixa orellana*)

It is hard to believe that the rich golden colour of butter and cheddar cheese does not originate from cream or milk but actually comes from a plant! The deep golden colour of smoked haddock also owes its hue to an annatto extract! The orange-yellow colour is extracted from the seed coats of a tropical tree, *Bixa orellano*. The tree has heart-shaped leaves and small pink to white flower bearing pods (Figure 5.1).

Figure 5.1 Leaves, pods and blossoms of *Bixa orellana*[1]

These pods, on maturing, contain numerous rust-coloured seeds. The resinous material with the same colour surrounding the seed is the source of annatto. The chemical name for the principal colour of annatto is bixin. Inside the prickly pods are about fifty small seeds covered with more dye. On touching a seed the red colour gets onto one and on everything one touches (Figure 5.2).

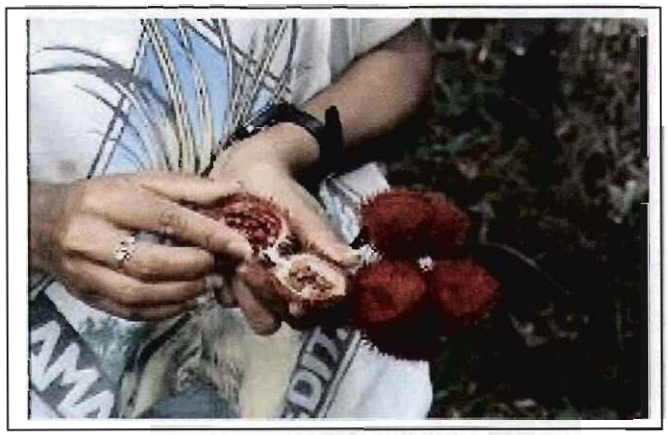


Figure 5.2 Opened pods showing rust coloured seeds

Bixa orellana is a native of the Amazon jungle and is a profusely fruiting tree that grows 5 to 10 meters in height. Throughout the rainforest, the indigenous tribes have used annatto seed as a body paint and a fabric dye.[2, 3] Annatto has been traced back to the ancient Mayan Indians who employed it as a principal colouring agent in foods, for body paints and as a colouring agent for arts, crafts and murals.[4]

5.1 Viable products

Annatto is available commercially in oil-soluble and water-soluble forms, depending on the method of extraction and subsequent preparation into dilutions, suspensions, mixtures, emulsions and powders. It is usually purchased on the basis of bixin content.

5.1.1 Oil soluble products

The oil-soluble form is prepared by softening the seeds with steam and extracting the pericarp with ethanol, a chlorinated hydrocarbon or a vegetable oil. Bixin, the major chromophore extracted, can make up more than 80% of the seed coat material. It can be crystallised out and is available in several concentrations as a crystal powder. It is only sparingly soluble in oil, up to 0.1 - 0.3 % by mass. The ingredient is pH sensitive, changing from yellow-orange to a pink shade at low pH, which has no effect on colour stability, though. Bixin is stable below 100 ^{0}C, but breaks down rapidly above 125 ^{0}C. Exposure to air is not a problem, but bixin behaves much like any other carotenoid by fading in the presence of light.

Figure 5.3 *Bixa orellana* growing throughout South and Central America, the Caribbean and some parts of Mexico

5.1.2 Water soluble products

The water soluble form is prepared by extraction of the softened pericarp with propylene glycol containing potassium hydroxide. In this system the bixin is saponified during extraction into norbixin. The latter is water soluble and solutions containing more than 5% can be achieved. Norbixin is known to precipitate to products high in calcium, with high acidity, or at very cold/frozen temperatures. It reacts with protein, with a slight shift to a delicate peach-red colour, sometimes seen in cheeses coloured with annatto. This binding property is useful in colouring products that should hold colour and not bleed into the surrounding medium. In products where norbixin is bound with protein or starch, it is stable to both light and heat. Norbixin solutions can be spray-dried to form a fine free-flowing powder.

The concentration of norbixin powder can range up to 15%. In this form, annatto is prone to oxidation with measurable loss of colour value over time. Aqueous solutions of norbixin, initially developed for the cheese industry, vary in concentration and are often identified as single strength (1.25-1.40 %), double strength (2.5-2.8 %), and triple strength (3.0-3.8 %). As noted earlier, some forms of annatto contain potassium hydroxide, a strong alkali.

5.2 Uses

Annatto has found use in dairy products, margarine, fats, popcorn oil, butter mixes, baked goods, icings, snacks, ice cream, salad dressing, yogurts, drinks, salmon/tuna, meat products, desserts, and dry mixes.

It is used in hair and skin care products, rouges and blushes. It has been used in direct compression tablets and tablet coating.[5]

Ethanol extracts of both the dried annatto fruit and the leaves were shown to have *in vitro* activity against *Escherichia coli* and *Staphylococcus aureus*. An aqueous extract of the root was shown to have hypotensive activity in rats and smooth muscle relaxant activity in guinea pigs, and a chloroform extract of dried seed was shown to have hypoglycemic activity in dogs.[6]

Annatto oil is extracted from seed and is the main source of the carotenoids, bixin and norbixin. Although its use as a food colour is well established worldwide, current trends show that it is being used increasingly in body care products. Annatto oil is emollient and its high carotenoid content provides antioxidant properties.[7] In body care products, annatto oil provides antioxidant benefits while adding a rich sunny colour to creams, lotions and shampoos.

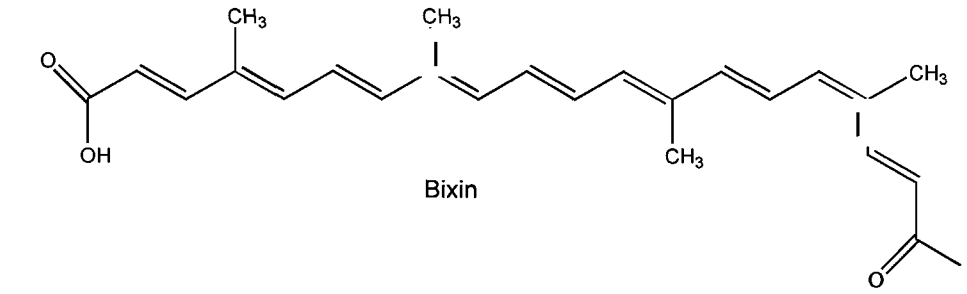
5.3 Chemical composition and structure

5.3.1 Chemical composition

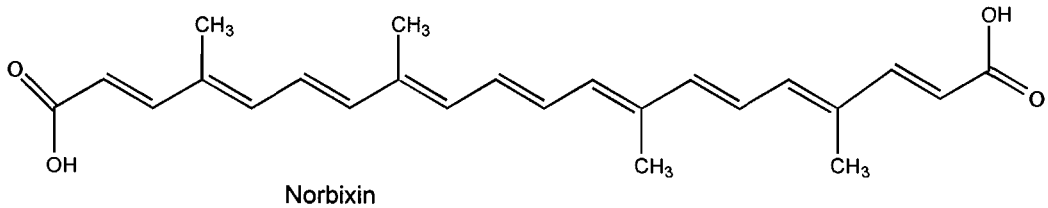
Annatto seeds contain 40-45 % cellulose, 3.5-5.5 % sucrose, 0.3-0.9 % essential oil, 3 % fixed oil, 4.5-5.5 % pigments, 13-16 % protein, as well as α- and β-carotenoids and other constituents.[8, 9, 10, 11] It also contains tannins, ethereal oils, saponins, mustard oil-like substances and mono- and sesquiterpenes.[12]

5.3.2 Structure of the main carotenoids

The main carotenoids found in *Bixa orellana* are bixin and norbixin[13], the structures of which are given below.



Bixin



Norbixin

5.4 Extraction experiments

Initial work carried out on annatto made use of the knowledge that at 350 atm and 60°C the product obtained using sc-CO_2 resembled most closely the organic solvent extract of the natural product under investigation. Later work was conducted on the micro-scale supercritical extractor (modified HPLC, see Chapter 3) to investigate the effect of ethanol and water as entrainers, the concentrations of which are being recorded as g entrainer per 100 g CO_2 used. These adjustments were made in order to study the effect of the natural moisture as well as the petroleum ether soluble fraction (60-80) i.e. the non-polar light oils, on the extraction efficiencies.

5.5 Extraction results

The results of the raw material analyses (in order to know the expected product yields if extracted exhaustively), the values of the extraction parameters used and the real extraction yields (%) obtained for annatto are presented in Tables 5.1, 5.2 and 5.3, respectively.

Table 5.1 Raw material analyses for annatto

Batch No.	Moisture (%)	Water (g)	Oils (%)	Oils (g)	Pigment (%)	Pigment (g)
1	10.1	0.810	4.42	0.355	3.56	0.286
2	10.3	0.690	4.25	0.284	3.58	0.240
3	10.1	0.661	4.64	0.305	3.49	0.229
4	11.2	0.747	4.47	0.297	3.51	0.234
5	11.9	0.777	4.41	0.287	3.55	0.231
6	11.2	0.734	4.48	0.293	3.54	0.232
7	11.9	0.823	4.65	0.322	3.51	0.242
8	11.6	0.817	4.35	0.307	3.52	0.248
9	9.96	103	4.26	44.1	3.55	36.8
10	11.3	139	4.00	49.2	3.57	43.9
	11.0 ± 0.8		**4.39 ± 0.19**		**3.54 ± 0.03**	

Table 5.2 Extraction conditions for annatto

Batch No	Batch (g)	EtOH (g)	CO_2 flow rate (g/h)	T (°C)	p (atm)	t (h)	CO_2 (g)	ρ CO_2 (g/mL)
1	8.03	0	5	25*	260	2.0	11	0.95
2	6.70	0	17	50	180	2.5	43	0.75
3	6.56	0	41	60	300	3.8	155	0.83
4	6.65	0	4	75	90	2.3	10	0.18
5	6.51	0	12	30*	90	6.0	71	0.70
6	6.54	2	88	40	210	3.0	27	0.39
7	6.91	2	2	80	320	1.9	4	0.77
8	7.05	2	10	55	110	3.0	32	0.40
9	1036	0	1096	85	500	18.0	20270	0.55
10	1230	0	114	65	300	29.0	3202	0.84

* subcritical temperature

Table 5.3 Extraction yields for annatto

Run	Water (%)	Water (g)	Oil (%)	Oil (g)	Pigment (%)	Pigment (g)
1	22.5	0.182	69.7	0.247	29.5	0.084
2	4.82	0.033	35.2	0.100	73.4	0.176
3	0.750	0.005	54.0	0.165	45.6	0.104
4	26.4	0.197	63.6	0.189	63.7	0.149
5	19.7	0.153	22.7	0.065	34.2	0.079
6	33.5	0.246	51.4	0.151	27.5	0.064
7	46.3	0.381	*	*	96.1	0.233
8	20.8	0.170	*	*	17.5	0.043
9	80.7	83.1	41.5	18.3	71.1	26.2
10	26.4	36.7	49.0	24.1	35.7	15.7

* oil content not determined

5.6 Optimisation

The first step in optimisation requires mathematical modelling using multiple regression analysis.[14, 15] This establishes the relationship among the various parameters used in the experimental runs. The best fit for the multivariant analysis was obtained from a linear regression curve fitting analysis, which means that an equation of the form

yield of pigment = a (batch size) + b (moisture content) + c (oil content) + d (pigment content) + e (amount of modifier) + f (CO_2 flow rate) + g (temperature) + h (pressure) + i (time of extraction) + intercept

is established, where the parameters in brackets are taken in the units in which they are listed in the tables above and where a, b, c, ... i are the regression coefficients obtained from the multidimensional data fit. The intercept can be considered to be the contribution of other less significant parameters such that a mathematically exact equation is obtained. By fiitting the data this way, no physical significance can be assigned to the magnitude of the regression coefficients, since they are simply such that an exact mathematical equation is obtained. The simple summation of the contributions of the different parameters can be statistically justified by assuming that the relatively small changes in yield brought about by relatively small changes in any of the contributing parameters may as a first approach be linear, an approach that is supported by a capable applied mathematician consulted in the course of this investigation. The importance of fitting the data this way is that the effect of all parameters, however small, is taken into account and that not only one or two major parameters (e.g. pressure and temperature) are solely considered to be process determining.

The second step in optimising extraction results uses the "goal seek" program of Microsoft Office 2000[16] while using the mathematical model established by the multiple linear regression techniques in the first step. This allows one to find the maximum efficiency of extracting a particular ingredient by keeping all others constant and varying only those parameters having the largest positive influence on the result. Alternatively, one can set the extraction efficiency in terms of yield of product (annato pigment in this case) at 99%, calculate the optimised yield of a given ingredient by keeping all other factors constant and compare this estimated yield to the real experimentally obtained yield. Tables 5.4-5.6 presents a summary of "goal seek" results for the existing parameters but changing only one parameter at a time.

Table 5.4 Comparison between actual and "optimised" moisture content for a pre-set extraction efficiency of 99%

"Optimised" moisture (%)	**Actual moisture (%)**	**Difference (%)**
11.53	10.08	1.45
10.84	10.31	0.53
11.18	10.07	1.11
11.97	11.23	0.74
13.29	11.94	1.35
12.70	11.21	1.49
11.97	11.91	0.06
10.54	9.96	0.58
12.59	11.27	1.32
13.30	11.59	1.71

Table 5.5 Comparison between actual and "optimised" oil content for a pre-set extraction efficiency of 99%

"Optimum" Oil Content (%)	**Actual Oil Content (%)**	**Difference (%)**
3.63	4.42	-0.79
3.87	4.25	-0.38
4.01	4.64	-0.63
4.03	4.47	-0.44
4.06	4.41	-0.35
4.09	4.48	-0.39
4.09	4.65	-0.56
4.27	4.26	+0.01
4.33	4.00	+0.33
4.64	4.35	+0.28

Table 5.6 Comparison between actual entrainer added and "optimised" entrainer added for a pre-set efficiency of 99%

"Optimum" Entrainer added (g/g of CO_2)	Actual Entrainer added (g/g of CO_2)	Difference
-2.13	0	-2.13
-0.79	2	-2.79
-1.64	0	-1.64
-1.09	0	-1.09
-1.99	2	-3.99
-0.19	0	-0.19
1.91	0	1.91
-0.86	0	-0.86
-1.94	0	-1.94
-0.52	2	-2.52

5.7 Concluding remarks

Although the data fitting procedure adopted here is possibly an over-simplification and that more advanced methods,[17, 18] such as dimensionless grouping of variables, need to be employed, the simple algebraic addition of the contributions of the different parameters has at least some merit, especially where the range of variation of parameters is not too large so that a linear response to a change in a given parameter can be anticipated. The regression equation for extraction of annato turned out to be

yield of pigment = 0.038 (batch size) + 48.0 (moisture content) - 170 (oil content) + 142 (pigment content) - 32.6 (amount of ethanol) - 41.9 (CO_2 flow rate) + 1.24 (temperature) + 0.384 (pressure) - 8.55 (time of extraction) - 327

with the parameters in brackets in the units in which they are listed in Tables 5.1 and 5.2.

The sc-CO_2 extraction of annato occurs as a result of chemical dissolution in the fluid since, as shown in Figure 5.4, the % yield exponentially increases at liquid-like densities ($0.8 < \rho < 1.0$ g/mL) where sc-CO_2 has solvent strengths comparable to solvents. The conclusion that the mechanism of extraction of annato is chemical dissolution, is supported by the magnitude of the activation energy derived from the Arrhenius plot in Figure 5.5 based on the temperature dependence of the extraction yield.

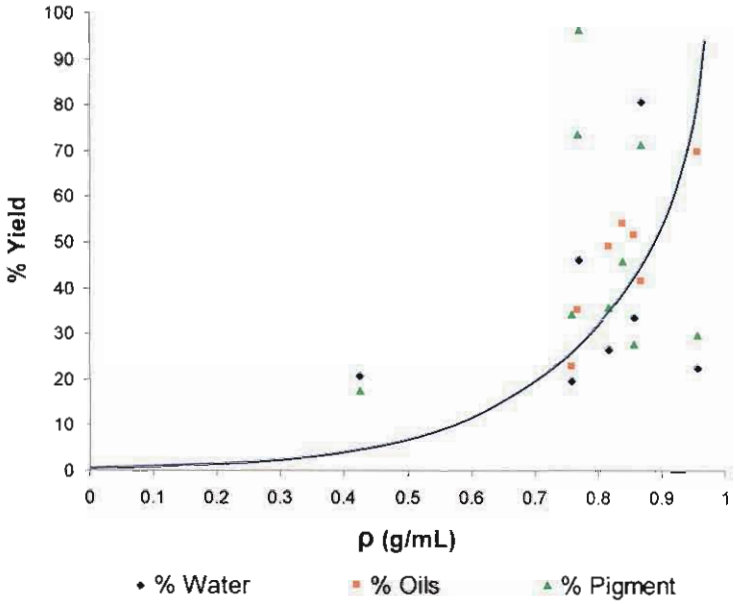


Figure 5.4 Dissolution of annato in sc-CO_2

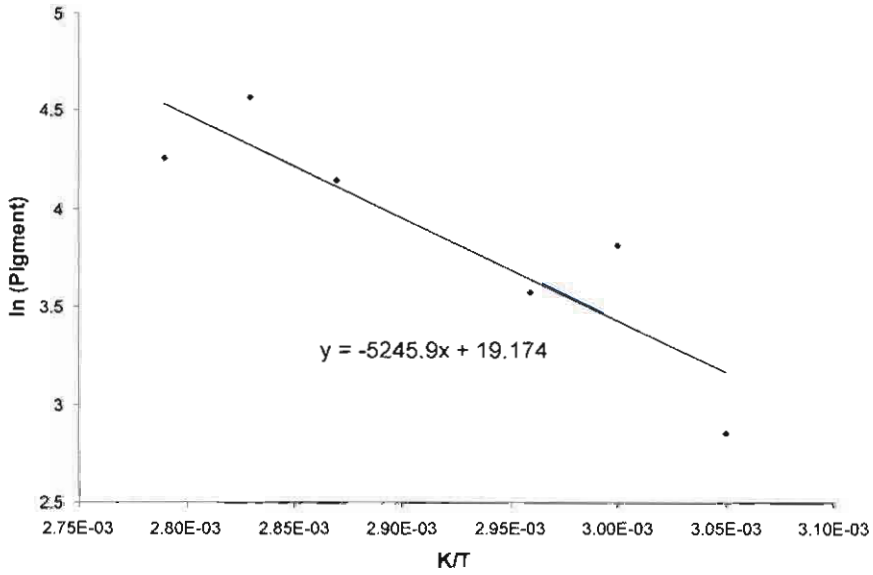


Figure 5.5 Arrhenius plot based on temperature dependence of extraction yield

The activation energy is calculated as E_a = (5 246 K^{-1})(8.31 x 10^{-3} kJ K^{-1} mol^{-1}) = 43.6 kJ mol^{-1}. This is a typical value to be associated with a process chemical in nature as opposed to a process physical in nature for which a value of E_a < 10 kJ mol^{-1} is expected.

References

1. www.raintree.com
2. Schultes, R., Raffauf, R., 1990, The Healing Forest, Dioscorides Press.
3. Acero, D. Prinipales Plantas Utiles de la Amazonia Columiana, Proyecto Radargrametrico del Amazonas, Bogata, 1979.
4. Heinerman, John, 1996. Heinerman's Encyclopedia of Healing Herbs & Spices. USA.
5. ColourMaker - 3345 E. Miraloma Ave., Suite 131 - Anaheim, California 92806 Phone 714-572-0444inquire@colourMaker.com
   FAX 714-572- 0999.
6. Rosita Arvigo and Michael Balick, 1993. Rainforest Remedies, One Hundred Healing Herbs of Belize. Lotus Press, Twin Lakes, WI.
7. Campelo, C.R., Contribuicao ao Estudo das Plantas Medicinais no Estado de Alagoas III, VII Simposio de Plantas Medicinais do Brasil, p. 85m 1-3 de Setembro, Belo Horizonte-MG, 1982.
8. Kember Mejia and Elsa Reng, 1995. Plantas medicinales de uso popular en la Amazonia Peruana. AECI and IIAP, Lima, Peru.
9. Ohasi, E.Y., et al, Urucu. Uma opcao para o Estado do Para. Belem. Secretaria 11 de Estado de Agricultura, Boletim Informative No. 6. 25 p. 1982.
10. Matos, F.J., Cienc. E Cult. 39: (7) Supl: 533. (1987).
11. Bressani, R., el al., Arch. Latinoam. Nutr. 33: (2): 356-376, (1983).
12. Rosita Arvigo and Michael Balick, 1993. Rainforest Remedies, One Hundred Healing Herbs of Belize. Lotus Press, Twin Lakes, WI.
13. Natural Colours from Botanicals, Anthony C. Dweck B.Sc., C. Chem., F.R.S.C., F.L.S., F.R.S.H.
14. Non-linear Regression Programs available from http//nlreg.com
15. Microsoft Office 2000 Professional, Excel Data Analysis.
16. Microsoft Office 2000, Excel "Goal Seek".
17. Giordano, F.R.; Weir, M.D. A First Course in Mathematical Modeling, Brooks/Cole Publishing Company (California), 1985, 228.
18. Giordano, F.R.; Weir, M.D. Example 2, How Long Should You Roast A Turkey?, In: A First Course in Mathematical Modeling, Brooks/Cole Publishing Company (California), 1985, 249-251.

Chapter 6

Pepper Extraction

Black pepper corns consist of the unripened fruit of *Piper Nigrum Longum* harvested and dried at moderate temperatures. Drying of the mature but still green berries is done in the sun for 3 to 4 days. Before drying, the green unripe berries (Figure 6.1) are separated from the stalks by hand-operated or motorised threshers. These are then spread out in a thin layer on rattan mats, which can be rolled up easily and taken indoors when necessary.

Figure 6.1 Unripe (green) peppercorns (left) and dried peppercorns (right)

After thorough drying, the berries change colour to a deep mahogany brown or black due to an enzymatic reaction similar to that occurring in tea leaves. This reaction can be prevented by drying unripe pepper fruit quickly at high temperatures e.g. heating over fire, pickling in brine or lyophilisation, i.e. drying in vacuum. The resulting product keeps its green colour. Green pepper is less pungent but very aromatic.

Alone or in combination with other spices, pepper is much loved for spicy meat stews, steaks, sauces and all kind of vegetable dishes. It may be cooked for quite a long time without losing its flavour. White pepper is sometimes preferred for light sauces, but generally has no advantage, unless pungency is preferred over fragrance (which might be the case for sweets). Green pepper gives a strong

aroma and is appropriate when the heavy pungency of black pepper might spoil a delicately flavoured dish.

6.1 History

Piper Nigrum Longum is thought to have originated in the hills of south-western India, where it occurs in wild condition from North Kanara to Kanyakumari. It is cultivated in Assam, Karnataka, Maharashtra and Kerala, and has not been known in Europe before Alexander the Great attempted to conquer India in c.a. the 4th century BC. Romans were said to have held black pepper in such high esteem that they paid their rents, dowries and taxes using individually counted peppercorns as currency instead of coins.

By the end of the 7th century, spices such as pepper, cinnamon and ginger had reached England. The middle ages saw pepper attaining considerable importance, and spice merchants became known as "Pepperers" in England, "Poivres" in France, and "Pfeffersacken" in Germany. It entered traditional cooking styles in Europe and Latin America, and became prominent in several Arabic spice mixtures. In the 15th century, increasing demand for pepper led to the age of exploration for Portuguese seafarers.

6.2 Cultivation

Pepper is a branching, climbing, perennial shrub with stout branches, trailing and rooting at the nodes. The leaves are 12.5-17.5 cm long and 5.0-12.5 cm wide. Pepper flowers are minute, borne in spikes, usually bearing male and female flowers on separate plants, but female plants often bear anthers and male plants a pistillode. The fruiting occurs in spikes, which vary in length and robustness. The pepper tree flowers in the rainy season, and produces fruit in autumn.

Figure 6.2 Cultivated pepper vines in India

About thirty species of *Piper Nigrum Longum* are known in India, of which only a few are known to be cultivated. Some of the most popular commercial varieties are listed in Table 6.1.[1]

Table 6.1 Some cultivated varieties of pepper

Name of Cultivar	Green berry yield per vine (kg)	Remarks
Kalluvalli	1 - 5.6	Hardy, drought and wilt resistant
Balamcotta	3 - 4.5	Dominantly bisexual, regular and heavy producer
Karimunda	No figures available	Early bearer but short lived
Malligesara	No figures available	Regular and heavy producer
Bangka	6 - 8	Susceptible to root wilt
Bangka/Kuching	18 – 27	Highly susceptible to root wilt

6.3 Other uses of pepper

In modern Indian medicine,[2] pepper is employed as an aromatic stimulant, in cholera weakness following fevers, vertigo, coma, etc., as a stomachic in dyspepsia and flatulence, and as an antiperiodic in malarial fever. It is also used as an alternative in paraplegia and arthritic diseases. Externally, it is valued for its rubefacient properties and as a local application for relaxed sore throat, piles and some skin diseases.

Pepper is pharmacologically[3, 4, 5, 6] used as an anti-allergic agent. The active ingredient, piperine, strongly inhibits hepatic aryl hydrocarbon hydroxylase and UDP-glucuronyl transferase activities, thus prolonging hexabartital sleeping time and zoxazolamine paralysis time in mice. Pepper oleoresin is also used as a natural insecticide and has been found to be as effective as the pyrethroids.

The cullinary uses of pepper are too numerous to mention. There is a US patent which claims to have found the secret of enhancing all food flavours by using pepper.[7] Piperine is also used to flavour brandy, the burning sensation being the direct result of the piperine content. The Afrikaans word "brandewyn" describes brandy beautifully as a wine that burns literally and figuratively. Extracts of pepper are used in self-defence sprays, also known as pepper sprays.

6.4 Chemical composition

Black pepper contains about 3% essential oil, the aroma of which is dominated by monoterpenes (up to 80%), such as sabinene and α-pinene, terpenes, β-pinene, myrcene, limonene, δ-3-carene and monoterpene derivatives (borneol, carvone, carvacrol, 1,8-cineol, linalool). Sesquiterpenes make up about 20% of the essential oil consisting of β-caryophyllene, humulene, β-bisabolone and caryophyllene oxide and ketone. Phenylether (eugenol, myristicine, safrol) are found in traces. Loss of monoterpenes due to bad storage conditions (especially for ground pepper) should be avoided.

The pungent principal in pepper is the alkaloid analogue compound piperine ($C_{17}H_{19}NO_3$, mole mass = 285.3 g mol^{-1}); it is the amide of 5-(2,4-dioxymethylene-phenyl)-hexa-2,4-dienoic acid (piperinic acid) with azinane (piperidine); only the *trans,trans* conformation contributes to pepper's pungency. Several piperidine analogues have been isolated from black pepper where the acid carbon backbone is partially hydrogenated (piperanine) or two carbon atoms longer (piperettine); amides of piperinic acid with pyrrolidine (piperyline) or isobutylamine (piperlongumine) have also been isolated. The total content of piperidine analogues in black pepper is usually about 6%.

A typical chemical composition of pepper comprises essential oils (3 %), piperine (6 %), starch (35-40 % in black pepper and 53-58 % in white pepper), fiber (14 %), moisture (10 %), Kjeldahl nitrogen (3 %) and non-volatile oils (7-12 %).

Table 6.2 Composition of fatty acids in pepper non-volatile oils determined by GLC

Fatty Acid	**% of Total**
Lauric	-
Myristic	-
Palmitic	16–32
Stearic	-
Oleic	18–29
Linoleic	25–35
Linoleinic	8–19
Arachitic	3-6
Behenic	3–6
Ligoceric	3–6

6.5 Extraction experiments

Extraction experiments carried out on pepper were based on initial work[8] showing that at 350 atm and 60°C the product obtained by sc-CO_2 extraction most closely resembled the organic solvent extract of the natural product under investigation.

The piperine content of each sample was determined by raw material analysis and used as a benchmark figure against which the amount of piperine extracted by sc-CO_2 could be expressed as a percentage. The water and light oil content of each sample were also determined as percentages of the raw material, but the yields of these components obtained by sc-CO_2 extraction were expressed both as percentages and as g of substance per 100 g of CO_2. There were two reasons for this. Firstly, yields of substances extracted by sc-CO_2 are often expressed this way. Secondly, these components may then be considered as "internal" entrainers and their effect on the extraction of piperine compared to the effect of added ethanol as an "external" entrainer.

The raw material analysis is presented in Table 6.3, and the extraction parameters and yield of piperine extracted using sc-CO_2 as extractant are presented in Table 6.4.

Table 6.3 Raw material analysis of black pepper

Batch No	% Water	% Oils	% Piperine
1	11.5	10.0	6.40
2	11.3	9.96	6.41
3	10.9	9.88	5.77
7	11.6	9.87	6.44
8	11.6	9.93	6.42
9	11.0	10.1	6.42
10	10.6	9.50	5.50
Average %	**11.2 ± 0.4**	**9.89 ± 0.19**	**6.19 ± 0.39**

Analysis was performed on ten different batches of material to warrant acceptable average values for the natural moisture or water content, the non-polar light oils, i.e. petroleum ether soluble fraction (60–80), and the piperine content of the material to be used in the sc-CO_2 extraction runs.

Table 6.4 sc-CO_2 Extraction of black pepper

No	Batch (g)	H_2O (g)	Oils (g)	Pipe-rine (g)	p (atm)	T (°C)	CO_2 (g/h)	t (min)	Piperine Extracted	
									(g)	(%)
1	6.95	0.797	0.630	0.445	350	80	53.2	100	0.273	61.3
2	7.34	0.832	0.731	0.470	240	35	42.0	260	0.0324	6.89
3	242	26.3	23.9	14.0	500	100	1917	1080	13.9	99.3
4	238	39.1	12.7	4.19	500	30	1917	4140	3.46	82.6
5	871	140	46.8	15.3	400	80	2556	1050	9.70	63.4
6	170	20.7	10.4	5.83	350	66	1643	1380	4.82	82.7
7	7.06	0.821	0.697	0.455	280	45	22.5	195	0.0345	7.58
8	5.80	0.671	0.576	0.372	300	90	32.1	160	0.0356	9.57
9	7.25	0.800	0.729	0.465	310	75	18.6	185	0.0628	13.5
10	7.25	0.766	0.689	0.399	300	60	37.6	280	0.390	97.7

6.6 Optimisation

As in all work done in this investigation, the extraction conditions were optimised by mathematical modelling using multiple linear regression analysis.[9] This established the relationship between the various parameters in the experimental runs. The best fit for the multivariant analysis was obtained from a linear regression fit to an equation of the form

yield of piperine = a (batch size) + b (water content) + c (oil content) + d (piperine content) + e (added modifier) + f (CO_2 flow rate) + g (temperature) + h (pressure) + i (time of extraction) + intercept

where the parameters in brackets are expressed in the units in which they are listed in Tables 6.3 and 6.4 and where a, b, c, ... i are the regression coefficients obtained from the multivariant data fit. The intercept is considered to be the contribution of other less significant parameters such that a mathematically exact equation is obtained. By fitting the data this way, no real physical significance can be assigned to the magnitude or sign of the regression coefficients, since the contribution of the various parameters have simply been added in the mathematical equation above. The simple summation of the contributions of the different role-playing parameters can be statistically justified by assuming that the relatively small changes in yield brought about by relatively small variations in contributing parameters may as a first approach be linear. The importance of fitting the data this way is that the effect of all parameters, however small, is taken into account and that not only one or two major parameters (e.g. pressure) are solely considered to be process or yield determining.

Table 6.5 Comparison between actual and predicted values for the extraction of piperine by sc-CO_2

Run	% Piperine Extracted	% Piperine Predicted
1	61.4	43.4
2	6.88	6.62
3	99.5	99.5
4	82.5	82.5
5	63.3	63.3
6	82.6	82.5
7	7.58	7.22
8	9.57	10.1
9	13.5	13.0
10	97.7	98.0

In Table 6.5 the actual results obtained in Table 6.4 are compared with the theoretical results predicted by the multiple linear regression equation

yield of piperine = -0.079 (batch size) -39.4 (oil/water ratio) + 14.8 (piperine content) + 0.092 (CO_2 flow rate) + 1.36 (temperature) - 0.468 (pressure) + 0.045 (time of extraction) - 37.6

by setting the yield of piperine as the unknown. The good comparison between the actual and predicted values creates some confidence in the method followed.

The second step in optimising the extraction results is to utilise the "goal seek" facility of Microsoft Office 2000[10] while using the mathematical model established by the regression analysis in the first step. This facility allows the maximum yield of a particular ingredient to be determined by keeping all parameters constant and varying only those having the largest effect. Alternatively, the yield of extracted piperine may be set at 99% and the optimised value of any given variable calculated by keeping all other variables constant. The estimated value can then be compared to the experimentally obtained value at the conditions concerned.

In order to obtain meaningful values and comparisons with regard to "internal" and "external" modifiers, the amounts of water and light oils present in the final extract were recalculated as g extracted per 100 g of CO_2 used. This made sense in view of the fact that the "external" entrainer (ethanol) had been premixed into the CO_2 by the supplier (Afrox Special Gases Division) to be 2 g ethanol per 100 g of CO_2 and that an assumption was made that the material in the separator represented the equilibrium of the gas and entrainers in the extractor. The recalculations are shown in Table 6.6 along with the extraction conditions and % piperine previously listed in Table 6.4.

Table 6.6 Extraction conditions for pepper adjusted to reflect amount of water and light oils in final extract as g of substance per 100 g of sc-CO_2

Extraction Conditions						Amount as g/100 g of CO_2			%
Run	**P (atm)**	**T (°C)**	**t(min)**	**g/hr CO_2**	**CO_2 (g)**	**Entr.**	**Water**	**Oils**	**Piperine**
1	350	80	100	53.2	5322	0	0.1799	0.0098	61.4
2	240	35	260	42.0	10907	0	0.1726	0.0009	6.88
3	500	100	1080	1917	2070360	0	0.0049	0.1320	99.5
4	500	30	4140	1917	7936380	0	0.0254	0.0881	82.5
5	400	80	1050	2556	2683800	0	0.0061	0.0966	63.3
6	350	66	1380	1643	2267340	0	0.0175	0.1183	82.6
7	280	45	195	22.5	4378	2	0.0088	0.0101	7.58
8	300	90	160	32.1	5130	2	0.2142	0.0208	9.57
9	310	75	185	18.6	3441	2	0.0160	0.2213	13.5
10	300	60	280	37.6	10534	2	0.0998	0.2415	97.7

It could be shown (Tables 6.7 and 6.8) that adjustments in the water content, for instance, brought about improvement in the efficiency with which piperine was extracted. Generally, less light oil fractions (non-polar material) and slightly more moisture (polar material) are required for improved extraction efficiency of piperine. The inclusion of these usually regarded less important process parameters in the regression equation therefore seems to be meaningful.

Table 6.7 Influence of water on extraction efficiency of piperine from pepper

g water per 100 g sample	0	2	4	6	8	11.5	14	20	30	52
% yield piperine extracted	31.0	33.6	36.8	38.9	41.5	46.2	49.4	57.4	70.5	99.5

Table 6.8 Influence of plant oils on extraction efficiency of piperine from pepper

g oils per 100 g of sample	0	6	12	16	20	24	28
% of piperine extracted	53.9	49.7	44.6	41.5	38.3	35.2	32.1

References

1 American Spice Traders Association, Pepper.

2 The Wealth of India, P.I.D., C.S.I.R., New Delhi.

3 Kholkute, et al., Ind. J. Exp. Biol., 1979, 17, 289-290.

4 George et al., J. Sci. Ind. Res., 1947, 6B, 42.

5 Satyavati, G.V., Ashok, K.G. and Tandon, N. eds. "Medicinal Plants of India ", ICMR, New Delhi. 1987, 2, 426.

6 Dhar et al. Ind. J. Exp. Biol., 1968, 6, 232.

7 US PATENT OFFICE Patent number 5536506.

8 Natural Product Extraction using sc-CO_2, Internal Report CG Smith Sugar, 1982, B. Clayton.

9 Microsoft Office 2000, Excel Program Data Analysis.

10 Microsoft Office 2000, Excel Program Goal Seek.

Chapter 7

Coffee Extraction

Coffee has become the world's most popular beverage. Eighty percent of all Americans drink coffee and consume more than 400 million cups daily.[1] The scandinavian countries are a close second, consuming more than 12 kg per capita.

"Coffee should be black as hell, strong as death, and sweet as love", a Turkish saying.

"Ah! How sweet coffee tastes! Lovelier than a thousand kisses, sweeter far than muscadel wine! I must have coffee...", JS Bach "Coffee Cantata".

Instant coffee has become extremely popular and is prepared by brewing the roasted ground coffee in the normal way with hot water. This "infusion" of coffee is then carefully evaporated by spray drying at high pressure, leaving only the coffee powder.

In some coffee products, coffee is replaced with chicory (a wildflower herb), fig, date, malt, or barley.

7.1 History

Although coffee is believed to have been grown near the Red Sea since the 7th century, an Arabian author of the 15th century, Shehabeddin Ben, wrote that Ethiopians enjoyed coffee ever since anyone could remember. By the 17th century, coffee plants were found throughout the Yemen region of Arabia. After a Turkish ambassador introduced it to the court of Louis XIV in 1779, Europeans quickly acquired a taste for it. A few years later, the Dutch introduced coffee to Java. In 1714, the Frenchman Desclieux planted a single cutting of a coffee tree

on the island of Martinique. Plantations soon grew from French Guiana to Brazil and Central America. Today, coffee is planted in moist regions around the world.

A more detailed history is included as an appendix to Chapter 7.

7.2 Coffee cultivation

Coffee is the seed of a cherry from the tree genus *Coffea*, a tree yielding about one kg of coffee per year. There are more than 25 species of coffee, the 3 main commercial types being Robusta, Liberia and Arabica, the latter representing 70 % of total production.

The bitter taste of cheap coffee is usually caused by the high caffeine content, and is typical of Robusta coffee which contains up to 3 % caffeine! Robusta is also higher in chlorogenic acid content than the other two types and produces the unpleasant "acid" feeling in the stomach of the consumer. Arabica coffee has in general less that 1 % caffeine as determined by the author.

Coffee cultivation is labour intensive. Currently the coffee industry employs more than 25 million people and is second only to oil in world trade.[1]

Figure 7.1 Coffee beans being hand-picked in Africa

Figure 7.2 Coffee beans dried in the sun

The coffee berries are usually hand-picked (Figure 7.1), washed, dried in the sun, (Figure 7.2), loaded onto trucks (Figure 7.3), sorted and then roasted (Figure 7.4) to produce the familiar coffee bean.

Figure 7.3 Coffee beans being hand loaded onto trucks

Figure 7.4 Familiar roasted coffee beans

7.3 Uses

Coffee is enjoyed as a beverage world-wide for its flavour as well as its stimulating effect. The stimulant of interest is the alkaloid caffeine, which is added to many soft drinks. Caffeine is addictive, and withdrawal symptoms are indicated by severe headaches.

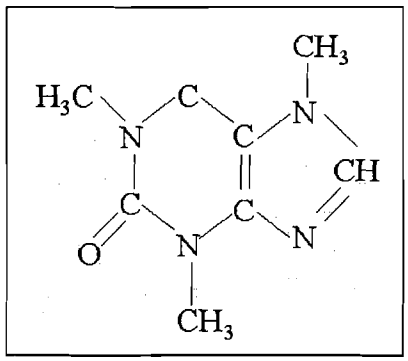


Figure 7.5 Molecular structure of caffeine (1,3,7-trimethylxanthine)

A cup of drip-brewed coffee has about 115 mg of caffeine, an espresso (and percolated coffee) about 80 mg and instant coffee about 75 mg. Decaffeinated coffee is not totally caffeine free, but contains about 3 mg of caffeine. A can of Coca-Cola has about 45 mg of caffeine, Pepsi-Cola 38 mg, Mountain Dew 54 mg and TAB 47 mg. A cup of tea has about 40 mg of caffeine, while 1 g of chocolate contains about 0.7 mg.

Originally caffeine had been removed from coffee by treating the green beans with chlorinated hydrocarbon solvents. Since 1980, commercial plants extracting caffeine from raw coffee beans have used carbon dioxide to decaffeinate coffee. This caffeine is then added to various beverages and medications.

Caffeine increases the power of aspirin and other painkillers. It is therefore found in some medicines. Caffeine withdrawal is one of the most common causes of severe headaches. Women who drink two or more cups of coffee a day, have an increased risk of developing osteoporosis. This, however, can be offset by drinking milk or yogurt to replace the lost calcium. Most studies have found that high caffeine consumption impairs fertility, and taken during pregnancy may cause premature or defected birth.

7.4 Coffee chemistry[2,3]

7.4.1 Carbohydrates

A wide range of carbohydrates, including polysaccharides and the low mol mass sugars (mono-, di- and tri-saccharides) are found in green coffee. Sucrose is the major free sugar present; arabica contains about 8% on a dry basis. Polysaccharides (glycans) amount to up to 50% on a dry basis of green coffee. Hydrolysis of coffee polysaccharides has been shown to give mannose, followed by galactose, then glucose and finally arabinose. On roasting coffee, major changes occur, depending on the degree of roasting, e.g. from light to dark, and simple sugars such as arabinose are progressively destroyed.

7.4.2 Nitrogenous components

These may be described in terms of three main groups of compounds: alkaloids, trigonelline together with nicotinic acid, and amino acids and proteins.

Caffeine

Caffeine is perhaps the best known and controversial alkaloid found in coffee and it is present at about 1-2% on a dry mass basis in arabica beans.

Conditions: column 20 x 2mm C18, flow 200 µL/min, 15 % acetonitrile/10mM phosphate buffer, detector UV 210 nm

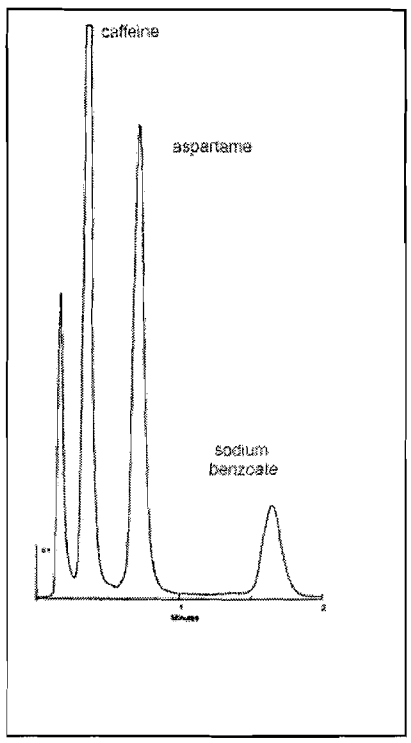


Figure 7.6 HPLC chromatogram of caffeine separation[1]

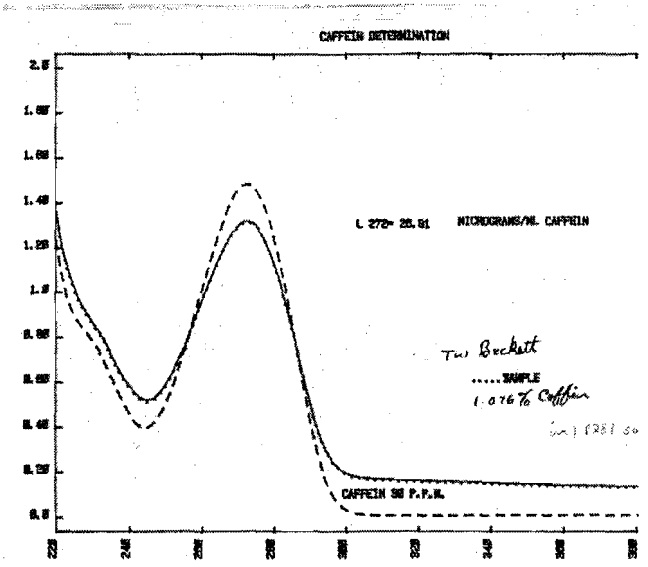


Figure 7.7 A typical UV spectrum of caffeine

Trigonelline

Trigonelline has received considerable attention as one of the nitrogen containing components of coffee. It is present at about 1% on a dry mass basis but it is thermally unstable and hence can lead to other nitrogenous materials, such as pyridines and pyrroles, upon roasting.

7.4.3 Chlorogenic acids

These are esters of quinic acid, between caffeinic acid and the C_5-OH group.

7.4.4 Volatile components

One of the difficult aspects of altering the composition of coffee during decaffeination is to ensure that the overall flavour of the coffee is not changed. Exactly which compounds are responsible for the sought after flavour of coffee still remains a mystery to this day. Early attempts of decaffeination made use of organic extracts of the roasted coffee bean. Coffee brewed by this technique was a tasteless brown stew! In order to try to find out more about these flavouring compounds, innumerable chemists analysed the vapours above roasted coffee. This analysis of the volatile material is usually achieved by initial separation using GC or HPLC. Headspace analysis techniques are employed, which involve sampling the vapour phase directly above the sample.

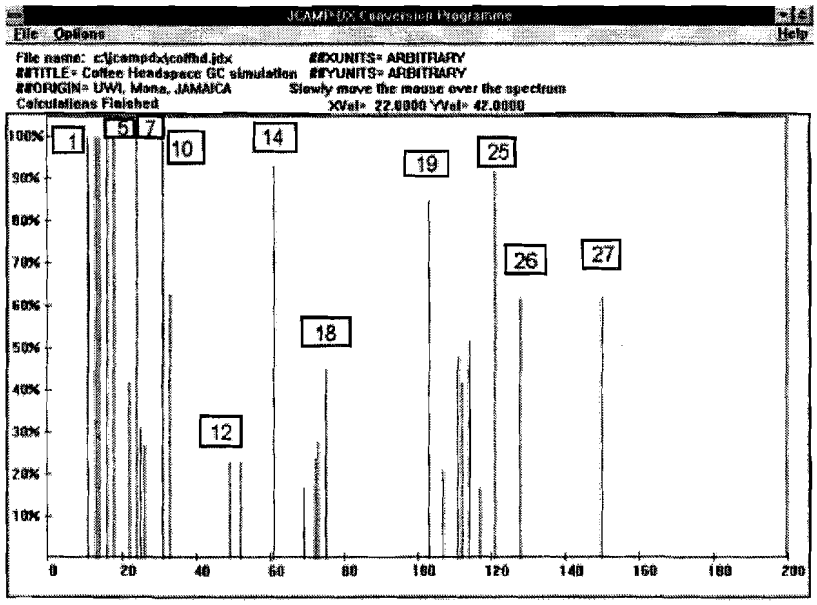


Figure 7.8: Typical chromatogram of the volatile components in roasted coffee.

Several of the more important peaks shown above have been identified as:

1. (NA), 2-methylfuran
2. (2170), 2-butanone
3. (NA), 2-methylbutanal
4. (NA), 2,5-dimethylfuran
5. (2370), 2,3-butanedione
6. (3523), pyrrolidine
7. (2841), 2,3-pentanedione
8. (NA), 2-methylthiophene
9. (3407), trans-2-methyl-2-butenal
10. (NA), 4-methyl-2,3-pentanedione
11. (NA), 3-methyl-1-hydroxybenzene
12. (NA), pyrazine
13. (NA), furfurylmethylether
14. (3309), 2-methylpyrazine
15. (2170), 2-butanol-3-one
16. (NA), 1-propanol-2-one
17. (3272), 2,5-dimethylpyrazine
18. (3273), 2,7-dimethylpyrazine
19. (2489), furfural
20. (NA), ethyleneglycol diacetate
21. (NA), furfuryl formate
22. (3173), 2-acetylfuran
23. (3387), pyrrole
24. (NA), 1-(2-furyl)-2-propanone

25. (2490), furfuryl acetate
26. (2702), 5-methylfurfural
27. (2491), furfuryl alcohol

The numbers in brackets are the FEMA (Flavor and Extract Manufacturers' Association of the USA)[4] codes. The list is endless, and we still do not know which ones are responsible for the sought after coffee flavour! Most of these volatile compounds are derived from pyrolysis or from reactions occurring during the roasting of the raw bean. These reactions, involving sugars, amino acids, organic acids and the phenolic compounds, give rise to the characteristic aroma and flavour associated with the different types of coffee.

The nature of the volatile compounds and the exact composition found is dependent on a variety of factors which include the location during growth (e.g. climate and soil conditions), storage of the beans (both during harvesting and subsequent to roasting) and the roasting conditions used (type of equipment, time and temperature). With GC-MS 81 pyrazine containing compounds, 15 pyridine derivatives and 28 oxazoles have been detected and identified.

7.4.5 Carboxylic acids

Aliphatic carboxylic acids play an important role in the quality of coffee and coffee infusions. Changes in pH can lead to ionisation of functional groups (e.g. phenolic hydroxy groups) and this can alter the flavour of the product. A number of acids reported to be present in coffee have characteristic flavours and their thresholds in aqueous solution may be as low as 10 ppm. 2-Methylvaleric acid, for example, is reported to impart a flavour of cocoa or chocolate, whereas pyruvic acid gives rise to a burnt caramel flavour.

In green coffee, non-volatile acids such as citric acid, malic acid, oxalic acid and tartaric acid make up less than 2%. In roasted coffee, over 30 aliphatic acids have been identified. These include 15 non-volatile mono-carboxylic acids C1-C10, while the remainder are volatile. In general, the darker the roast, the lower the acid content.

7.5 Extraction experiments

Although there is considerable interest in extracting the overall coffee flavour using *sc*-CO_2, the experiments performed for the purpose of this dissertation were limited to the extraction of caffeine. At the selected conditions of 300 atm and 60°C, the *sc*-CO_2 derived product contained almost everything that could be extracted. It also resembled most closely the organic solvent extract of the natural product under investigation.

The raw material analysis is presented in Table 7.1, and the percentages of moisture, oil and caffeine present in several samples were used to calculate the efficiency with which these components could be extracted by sc-CO_2.

Table 7.1 Raw Material Analysis

Batch No	% Moisture	% Oils	% Caffeine
1	9.94	16.0	4.00
2	9.46	12.4	4.05
3	10.3	18.7	4.08
4	8.97	20.4	4.42
5	10.5	20.0	4.42
Average	**9.83 ± 0.62**	**17.5 ± 3.3**	**4.19 ± 0.21**

A first ten extraction runs were performed to study the effect of ethanol as an entrainer using the micro scale supercritical extractor (Chapter 2). These experiments are detailed in Table 7.2.

Table 7.2 Conditions of extraction runs

Run	CO_2 flow rate (g/h)	Bulk density kg/m³	p (atm)	T (°C)	t (min) dynamic mode	ρ (CO_2) (kg/m³)	EtOH added g	sc-CO_2 extraction efficiencies (%) water	caffeine	oil
1	13.4	0.50	129	45	33	0.64	0	5.00		3.40
2	46.8	0.58	99	55	82	0.26	0	51.0	2.80	8.40
3	63.7	0.44	240	35	70	0.89	2		42.1	60.6
4	98.7	0.36	300	95	30	0.65	2		86.3	45.1
5	41.6	0.61	290	65	45	0.77	0	3.20	3.60	13.4
6	126	0.34	150	35	108	0.81	2	3.70	28.4	11.7
7	124	0.55	180	80	120	0.54	2	11.2	32.0	65.7
8	32.0	0.38	210	40	52	0.82	0	7.60	8.80	5.40
9	107	0.34	150	35	126	0.81	0	32.0	4.00	20.0

The prediction equation for the % caffeine extracted during the runs performed with the micro scale extractor can be written as

% caffeine = -21.7 (batch size in g) - 54.1 (bulk density in g/mL) + 10 325 (g of water in CO_2 phase) + 769 (g of oil in CO_2 phase) - 5.43 (% caffeine in raw material) + 0.718 (flow rate of CO_2 in g/h) + 0.397 (pressure in atm) - 0.395 (temperature in ^0C) - 0.0512 (dynamic time in min) - 61.0 (density of CO_2 in g/mL) - 25.3 (ethanol entrainer in g/100 g CO_2) + 54.7

Since the amount of ethanol entrainer added was recorded as g per 100 g of CO_2 used, the amount of water and of light oil fraction presented as a percentage of the raw material in Table 7.2 were recalculated in order to reflect them as entrainers in g per 100 g of CO_2 and to see their effect on the efficiency of extraction. These adjustments are presented in Table 7.3.

Table 7.3 Extraction efficiencies expressed as moisture, oil and caffeine content in g per 100 g of CO_2.

RUN No.	**Batch size (g)**	**Bulk density kg/m³**	**g water per 100 g CO_2**	**g oils per 100 g CO_2**	**g caffeine per 100 g CO_2**
1	3.22	0.50	0.215	0.260	
2	7.40	0.58	0.580	0.170	0.0136
3	2.83	0.44		0.404	0.0152
4	2.34	0.36		0.374	0.0458
5	3.90	0.61	0.0393	0.293	0.0180
6	4.34	0.34	0.0070	0.0392	0.0220
7	3.51	0.55	0.0156	0.163	0.0185
8	2.44	0.38	0.0657	0.0831	0.0324
9	4.36	0.34	0.0610	0.0679	0.0325

Clearly, not all the water, oil and caffeine present in the experimental sample dissolved in the sc-CO_2, but only the percentage given as % efficiency of extraction for the respective ingredient in Table 7.2. If these components truly were to act as additional entrainers, only the amount actually present in the sc-CO_2 should be taken into consideration and not the total amounts present in the sample. Table 7.3 reflects the actual amounts of these components in 100 g of the sc-CO_2 phase. In making use of the regression analysis used throughout this dissertation, the combined effects of these changes could be studied without investigating each separately. From the mathematics governing the prediction equation it is possible to study the individual effects of these parameters.

Continuing with the thought that the actual g of water and g of oil in the CO_2 phase could play an important role in the selectivity of the CO_2, a further regression analysis was conducted on the recalculated values in Table 7.3. When a regression analysis taking into account the amount of water, oil and caffeine expressed in g per 100 g of CO_2 was conducted, the following prediction equation was obtained:

% caffeine = 0.003 (batch size in g) - 72.3 (bulk density in g/mL) + 1.46 (g of water per 100 g CO_2) - 368 (g of oil per 100 g CO_2) - 1446 (g of caffeine per 100 g CO_2) - 0.023 (CO_2 flow rate in g/h) - 0.219 (pressure in atm) + 1.59 (temperature in ^{0}C) + 0.084 (dynamic time in min) + 113 (density of CO_2 in g/mL) + 6.4 (ethanol in g per 100 g CO_2) + 5.17

Additional runs were performed to study the effect of added water on the extraction results. These are documented in Table 7.4.

Table 7.4 Experimental runs with added water before and after extraction

Run No.	Batch (g) initial	Batch (g) final	H_2O initial (%)	H_2O initial (g)	Added H_2O (mL)	Total H_2O available (g)	final H_2O (%)	final g of H_2O	H_2O removed (g)
10	115	113.8	11.5	13.23	40	53.23	10.06	11.44	41.8
11	500	572.1	10.9	54.50	130	184.5	16.20	92.7	91.8
12	332.7	312.6	10.5	35.03	120	155.03	4.62	14.4	141
13	519.3	656	11.5	59.72	200	259.72	30.55	200	59.3
14	501	501	11.0	55.11	250	305.11	13.26	66.4	239
15	406	406	11.0	44.66	100	144.66	13.06	53.0	91.6
16	602	602	11.0	66.22	50	116.22	11.25	67.7	48.5
17	520.2	608.3	10.0	52.12	100	152.12	13.74	83.6	68.5
18	800	829	10.7	85.68	0	85.68	10.38	86.1	-0.37
19	322.6	320.6	11.8	37.97	0	37.97	9.77	31.3	6.65
20	519.2	604.6	8.3	42.99	50	92.99	12.73	77.0	16.0

By determining the water content of the sample prior to and after an extraction run (i.e. of the initial and final batch), it is possible to determine how much of the added water had been removed during extraction.

Finally, multiple linear regression analysis was conducted to include additional work on the contribution of static time, i.e. time during which the coffee beans were allowed to soak in sc-CO_2 using the static mode of extraction, and of charcoal added to the extractor in order to effect an equilibrium shift of the sample material from the sc-CO_2 to the charcoal. The details are listed in Table 7.5.

Table 7.5 Details of experimental runs in static mode and for added charcoal

Run No.	Dynamic Time (min)	Static Time (min)	Bulk Density (kg/m³)	Density CO_2 (g/mL)	Charcoal added (g)	Entrainer Ethanol (g/100g)	Extraction Efficiency of Caffeine (%)
1	33	0	0.50	0.64	0	0	0.0
2	82	0	0.58	0.26	0	0	2.8
3	70	0	0.44	0.89	0	2	42.1
4	30	0	0.36	0.65	0	2	86.3
5	45	0	0.61	0.77	0	0	3.6
6	108	0	0.34	0.81	0	2	28.4
7	120	0	0.55	0.54	0	2	32.0
8	52	0	0.38	0.82	0	0	8.8
9	126	0	0.34	0.81	0	0	4.0
10	360	0	0.26	0.71	0	0	13.0
11	480	0	0.23	0.71	0	0	22.2
12	750	0	0.31	0.71	0	0	23.2
13	0	4170	0.24	0.70	536	0	94.9
14	0	1200	0.23	0.65	500	0	57.4
15	0	360	0.19	0.85	602	0	43.5
16	0	228	0.22	0.85	402	0	36.1
17	0	4320	0.24	0.70	536	0	69.6
18	90	3990	0.36	0.88	0	0	13.6
19	120	90	0.30	0.91	0	0	2.5
20	0	5490	0.24	0.70	536	0	90.0

The regression equation then became:

% caffeine = -0.013 (batch size in g) - 50.4 (bulk density in g/mL) + 1.28 (g of water per 100 g CO_2) - 139 (g of oil per 100 g CO_2) - 1 027 (g of caffeine per 100 g CO_2) - 0.011 (CO_2 flow rate in g/h) - 0.15 (pressure in atm) + 1.24 (temperature in ^{0}C) + 0.006 (static time in min) + 0.059 (dynamic time in min) + 85.0 (density of CO_2 in g/mL) + 0.019 (charcoal added in g) -21.6.

The % error with such a vast number of parameters was relatively large (some 13%), though still acceptable statistically when taken into account analytical errors, instrumental errors and the fact that scaling-up from 10 g to 1 500 g took place during these experiments.

References

1 Internet Search (Google) "Coffee Statistics".

2 "Coffee" Volume 1: Chemistry. Edited by R.J. Clarke and R. Macrae, Elsevier Applied Science Publishers, London and New York, 1985.

3 Oskar Eichler, "Kaffee und Coffein", Vol 2, Springer Verlag Berlin, 1983.

4 Aldrich Flavors and Fragrances Catalog.

Appendix: The History of Coffee

Long, long ago — Members of the Galla tribe in Ethiopia noticed that they got an energy boost when they eat a certain type of berry, ground up and mixed with animal fat.

c.a. 700 A.D. — Arab traders brought coffee back to their homeland and cultivate the plant for the first time on plantations. They also began to boil the beans, creating a drink called "qahwa" (literally, "that which prevents sleep").

1453 — Coffee was introduced to Constantinople by the Ottoman Turks.

1475 — The world's first coffee shop, Kiv Han, opened in Constantinople. Turkish law made it legal for a woman to divorce her husband if he fails to provide her with her daily quota of coffee.

1511 — The sultan, Khair Beg, the corrupt governor of Mecca, tried to ban coffee for fear that its influence might foster opposition to his rule. He pronounced that coffee was sacred and had the governor executed.

1587 — Sheik Abd-al-Kadir wrote "no one can understand the truth until he drinks of coffee's frothy goodness."

c.a. 1700 — In Italy, Pope Clement VIII baptized and blessed coffee, thereby making it an acceptable Christian beverage!

1713 — The Dutch unwittingly provided Louis XIV of France with a coffee batch whose descendants would produce the entire Western coffee industry when in 1723 French naval officer Gabriel Mathieu de Cheu stole a seedling and transported it to Martinique. Within 50 years an official survey recorded 19 million coffee trees on Martinique. Eventually, 90 percent of the world's coffee spreaded from this plant.

1727 — The Brazilian coffee industry got its start when Lieutenant Colonel Francisco de Melo Palheta was sent by his government to arbitrate a border dispute between French and Dutch colonies in Guiana. Not only did he settle the dispute, he also struck up a secret liaison with the wife of French Guiana's governor. Although France guarded its New World coffee plantations to prevent cultivation from spreading, the lady said good-bye to Palheta with a bouquet in which she hid cuttings and fertile seeds of coffee.

1732 Johann Sebastian Bach composed his "Kafee-Kantate".

1745 First coffee-house opened in Italy.

1752 First coffee-house opened in England.

1775 England's King Charles II tired to suppress coffee-houses, supposedly because men were neglecting their families to discuss business and politics over coffee. His proclamation was revoked after public outcry.

1790 With a coffee plant smuggled out of the Arab port of Mocha, the Dutch became the first to transport and cultivate coffee commercially in Ceylon and in their East Indian colony Java, source of the brew's nickname.

1900 Hills Bros. began packing roast coffee in vacuum tins, spelling the end of the ubiquitous local roasting shops and coffee mills.

1901 The first soluble "instant" coffee was invented by Japanese-American chemist Satori Kato of Chicago.

1903 Coffee was decaffeinated for the first time. German coffee importer Ludwig Roselius turned a batch of ruined coffee beans over to researchers, who perfected the process of removing caffeine from the beans without destroying the flavour. He marketed it under the brand name "Sanka" (a combination of "sans" and "caffeine"). Sanka was introduced to the United States in 1923.

1938 Having been asked by Brazil to help find a solution to their coffee surpluses, the Nestle company invented freeze dried coffee. Nestle developed Nescafé and introduced it in Switzerland.

1947 In Italy, Achille Gaggia perfected his espresso machine. Cappuccino was named for the resemblance of its colour to the robes of the monks of the Capuchin order.

1971 Carnation introduced CoffeeMate non-dairy creamer, a power composed of corn syrup solids, vegetable fat, sodium caseinate, and various additives.

1980 The first commercially viable plant decaffeinating coffee using supercritical carbon dioxide was set up by "Kafee Hag" in Germany.

Chapter 8

Looking Back

The main hypothesis to be tested in this investigation was the viability to extract a selected few natural product ingredients by supercritical carbon dioxide (sc-CO_2). The extraction of annato (Chapter 5), piperine (Chapter 6) and caffeine (Chapter 7) could be successfully achieved by employing micro and pilot plant scale supercritical extractors.

The investigation revealed that there were more process determining factors that needed to be investigated than the few parameters normally considered. Through the completion of this dissertation it is hoped that more of the factors governing sc-CO_2 extraction may in future be better understood, thus enabling researchers to continue the quest for complete understanding of SFE.

The effect of added entrainers, such as water and ethanol, and of entrainers present in the natural raw material, i.e. moisture and light oils, was shown by virtue of experimental runs that formed part of the investigation. It was evident in all the work carried out on annatto, pepper and coffee that adjustments in the water content brought about change in the extraction efficiency. Generally, less of the light oil fraction (non-polar material) and slightly more moisture (more polar material) were required for optimum extraction efficiency.

Although there are many different available programs for the optimisation of experimental results, multiple linear regression analysis proved to be useful for processing the extraction results obtained for the three natural products investigated. The mathematical model could be used as a tool to decide which of the parameters are critical to achieve high extraction efficiency of the sought after substance. The correlation coefficient for the data fit for the various parameters was excellent in each case, with a percentage error of between one and two. The correlation between the experimentally obtained and theoretically predicted values was equally good. It is hoped that the optimisation techniques for sc-CO_2 will serve as guidelines for future investigations.

The acquisition of comparable extraction results using both micro and pilot plant scale extractors proved the ability to successfully upscale sc-CO_2 work. With the mathematical model applied to all the work done and a one hundred and fifty times scale up, a 13% regression statistical error was obtained. Usually, with more experimental results, this error can be reduced.

Finally, the investigation brought a meaningful close to experimental work initiated on the sc-CO_2 extraction of a number of natural product ingredients many years ago as a local introduction to supercritical technology and completed within a research group that currently specialises in this field.

Supplementary References

1. Dr. E. Lack, DI. G. Bunzenberger, Prof. DI. Dr. R. Marr, "Hochdruckextraktion mit CO_2", Interne Arbeitsitzung des GVC-Fachausschusses Thermische Zerlegung von Gas- und Flüssigkeitsgemischen, 9-10 Juni 1983, Kaiserslautern.

2 Dr. G. Bunzenberger, DI. E. Lack, Prof. DI. Dr. R. Marr, "CO_2-Extraktion - Vergleich der überkritischen Fluidextraktion mit der verflüssigten Betriebsweise anhand von Problemen der Naturstoffextraktion" VDI-Berichte Nr. 505, 1983.

3 Dr. E. Lack, DI. G. Bunzenberger, "Hochdruckextraktion: CO_2 als Lösungsmittel für die Naturstoffgewinnung" Österr. Chemie Zeitung, Juli/August 1983, 201, 202.

4 Dr. E. Lack, G. Bunzenberger, Prof. Dr. R. Marr, "Plants and Process Engineering for High-Pressure Extraction of Natural Products", International Solvent Extraction Conference, August 26-September 2, 1983, Denver, Colorado, USA.

5 Dr. E. Lack, G. Bunzenberger, Prof. Dr. R. Marr, "High Pressure Extraction of Natural Products with CO_2", 4th Italian-Jugoslavian-Austrian Chemical Engineering Conference, 24-26 September 1984, Grado, Italy.

6 Dr. E. Lack, DI. G. Bunzenberger, Prof. Dr. R. Marr, "Kriterien für die Auslegung von Hochdruckextraktionsanlagen", International Symposium, High-Pressure, Chemical Engineering, 8-10 Oktober 1984, Erlangen, BRD.

7 Dr. E. Lack, Prof. Dr. R. Marr, "Hochdruckextraktion von Raps mit Schwerpunkt auf Stoffaustauschuntersuchungen" GFD-Vortragstagung 9-12 September 1985, Göttingen, BRD.

8 DI. Dr. E. Lack, Prof. Dr. R. Marr, "Kriterien für die Auslegung von Hochdruckextraktionsanlagen unter besonderer Berücksichtigung des Stoffaustausches" 6. Österr. Chemietage 25-27 September 1985, Uni Linz, Austria.

9 Dr. E. Lack, Prof. Dr. R. Marr, "Estimation of the Process Parameter for High-Pressure Extraction of Natural Products" Fourth Symposium on Separation Science and Technology for Energy Applications, October 20-24, 1985, Knoxville, Tenessee, USA.

10 Dr. E. Lack, Prof. Dr. R. Marr, "Beschreibung des Lösungsmittelkreislaufes bei der Hochdruckextraktion mit CO_2, apparative und energetische Betrachtungen" Interne Arbeitssitzung der GVC-Fachausschüsse-Hochdruckverfahrenstechnik 10-11 April 1986, Erlangen BRD.

11 Dr. E. Lack, Prof. Dr. R. Marr, "Batch and Continuous Experiments in Supercritical Extraction Design Criteria, Mass Transfer and the Cycle Process", Chapter 9, 431-457, Theories and Application of Supercritical Gas Extraction N.T.S. (New Technology & Science, Tokyo), 1986.

12 Dr. E. Lack, H. Seidlitz, "High-pressure extraction by means of CO_2 outgrown of childhood", Process Engineering, Edition Europe June 1986, 16-17.

13 Dr. E. Lack, Prof. Dr. R. Marr, "Mass Transfer Problems in High-Pressure Extraction", ISEC 86, International Solvent Extraction Conference, 11-16 September 1986, München, BRD.

14 Dr. E. Lack, Prof. Dr. R. Marr, "Estimation of the Process Parameter for High-Pressure (supercritical fluid) Carbon Dioxide Extraction of Natural Products" Separation Science and Technology 23 (1-3), 63 - 76, 1988.

15 Dr. E. Lack, Prof. Dr. R. Marr, "Batch and Continuous Experiments in Supercritical Extraction, Design Criteria, Mass Transfer and the Cycle Process", International Symposium on Supercritical Gas Extraction, December 14-15, 1987, Meguro Gajeon, Tokyo, Japan.

16 Dr. E. Lack, H. Seidlitz, P. Toro, "Decaffeination of Coffee Samples by CO_2 Extraction", ASIC 13e (Association Scientifique Internationale du Café), 21-25 April, 1989, Paipa, Columbia.

17 Dr. E. Lack, H. Seidlitz, "Findings and Experience Acquired in Operating Industrial High-Pressure Extraction Plants with Supercritical CO_2" 2nd International Symposium High-Pressure Chemical Engineering, 24-26 September 1990, Erlangen, BRD.

18 Dr. E. Lack, H. Seidlitz, "Commercial scale decaffeination of coffee and tea using supercritical CO_2", Chapter 5 in "Extraction of Natural Products using Near-Critical Solvents" (eds. M B King and T R Bott), Blacky Academic & Professional (1992).

19 Dr. E. Lack, H. Seidlitz, "Extraction of Food Ingredients by means of fluid CO_2 at pressures higher than 500 bar", Third International Symposium on Supercritical Fluids, 17-19 October, 1994, Strasbourg, France.

20 H. Seidlitz, Dr. E. Lack, Y.C. Kalia, "Commercialization of SCFE Technology", Asian Symposium on Supercritical Fluid Extraction Technology for Natural Products, 27-29 Sept. 1994, IIT Delhi, India.

21 Dr. E. Lack, H. Seidlitz, "Extraction of Food Ingredients with supercritical CO_2", 9th World Congress of Food Science and Technology, July 30-August 4, 1995, Budapest, Hungary.

22 Dr. E. Lack, H. Seidlitz, "Basic Design Features, Application and Research", Internal lecture on request March 15, 1996.

23 H. Seidlitz, Dr. E. Lack, "Principles of CO_2 Technology and Application", "Consideration of Supercritical Fluid Extraction at Pressures above 500 bar", "Economical Considerations regarding Design of High-Pressure Extraction Plants." Lectures held at "Laboratory General d' Assaigs i Investigacions de la Generalitat de Catalunya", May 8, 1996.

24 Dr. E. Lack, H. Seidlitz, "Standardized Industrial Scale High-Pressure Extraction Plants", National Scientific Conference on "Analytical and Technological Use of Supercritical Fluids", May 16, 1996, Budapest, Hungary.

25 Dr. E. Lack, H. Seidlitz, "Application of Supercritical Fluid Extraction of Spices and Herbs with Pressures up to 800 bar", 3rd International Symposium on High-Pressure Chemical Engineering, 7-9 October, 1996, Zürich, Switzerland.

26 H. Seidlitz, Dr. E. Lack, "Separation of Essential Oils and Flavours from Spices and Herbs", Chemical Industry Digest, Fourth Quarter 1996 (December) 91-97.

27 E. Lack, H. Seidlitz, "Standardized industrial scale high-pressure extraction plants", The First European Congress on Chemical Engineering, 4-7 May, 1997, Florence, Italy.

28 H. Seidlitz, Dr. E. Lack, "CO_2 Extracts - Food and Medicine?" International Food Marketing & Technology, Vol. 11 No. 4, August 1997, 27-31.

29 H. Seidlitz, Dr. E. Lack, "Supercritical Fluid Extracts - Used in Food and as Medicine?", "Supercritical Fluid Extraction Symposium" in Taiwan National Cheng Hwa University, Taiwan Cheng Kong University, Taiwan.

30 Isolation and identification of new apocarotenoids from annatto (*Bixa orellana*) seeds. Publication Apr 1997, Analytical Abstracts.

31 Organic Chemistry Practical Manual, University of Natal.

32 AOAC Methods (1980).

33 M Verzde, J van Dyck and H Claus, "On the Analysis of Hop Bitter Acid", J. Inst. Brewing, Febr. 1980, Vol 86, 9-14.

34 American Spice Trader's Association, Paprika.

35 Hoffman, Patrick G.; Lego, Mary C.; and Galetto, William G. "Separation and Quantification of Red Pepper - Major Heat Principles by Reverse-Phase High-Pressure Liquid Chromatography" J. Agric. Food Chem. 1983, Vol. 31, 1326-1330.

36 Internet ref. 70431.3065@compuserve.com.

37 This method was cited as a reference in ASTM E-1083-88 "Standard Test Method for Sensory Evaluation of Red Pepper Heat" which uses a taste panel approach to test heat content of red pepper powder.

38 Hewlett Packard Application Notes "Separation of Carotenoids".

39 Jean Pierre Calame and Rudolf Steiner, CO_2 Extraction in the Flavour and Perfumery Industries, Chemistry and Industry, June, 1982.

40 Instrumental Methods of Chemical Analysis, Ewing, McGraw-Hill, 1975.

41 Extraction of annatto seed pigment [C. I. Natural Orange] by supercritical carbon dioxide, Publication Nov-Dec 1991 Analytical Abstracts.

43 http://facs.scripps.edu/surf/djones/coffee.html