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Extraction of Alkaloids from Natural Plants Using Supercritical Fluids

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I. INTRODUCTION

Alkaloids are nitrogen-based organic compounds commonly found in leaves, seeds, and roots of plants, particularly the Papaveraceae (poppy and opium), Papilonaceae (lupins), Ranunculaceae (aconitum), and Solanaceae (tobacco) families (1). Some alkaloids have bitter taste and are highly toxic, but can have therapeutic applications when used in moderate amounts. Almost all alkaloids have pharmacological activity together with pronounced physiological actions of interest to chemists, biochemists, and pharmacologists.

Alkaloids such as caffeine, morphine, cocaine, nicotine, quinine, codeine, emetine, pilocarpine, among others, form the active components in a variety of stimulants and medication drugs. Among the most consumed alkaloid-containing products are coffee, tea, and guaraná, which contain caffeine, tea and cocoa nuts, which contain theobromine; and tobacco, which contains nicotine. The alkaloids found in these products have stimulant and/or sedative effects.

Until recently, most of research on alkaloids has focused on the chemical aspects of their structure and isolation techniques (2). Increased attention is currently devoted to the identification of the pharmacological effects of these compounds on animals and the extraction and characterization of new alkaloids. The extraction of the very small quantities of alkaloids in natural plants required the development of new and improved experimental equipment and instrumental techniques.

Alkaloids are normally removed from natural products using solvent extraction. For the purification of these alkaloids in the extracts, conventional separation processes such as fractional distillation, fractional crystallization, and fractional salt precipitation (3) as well as the new and promising technology of extraction with supercritical carbon dioxide (4, 5) have been employed. The potential of each of these processes is determined by a compromise between cost and selectivity. For large production volumes, the tendency is to choose the conventional processes mentioned above, as they only require conventional equipment. However, these processes bring with them the risk of contamination of extracted products with residual chemical solvents used in extraction and possible alteration in the quality of thermally labile products due to the high temperatures used in distillation. Extraction with supercritical carbon dioxide requires higher capital costs but is highly selective; therefore, it is recommended for the extraction and purification of valuable products such as alkaloids. With this technology, extraction and purification are carried out simultaneously since solvent power and selectivity of the supercritical fluid is easily modified with changes in thermodynamic variables of temperature and pressure and the use of a suitable cosolvent. In supercritical carbon dioxide extraction, there is no risk of thermal degradation as the extraction is carried out at temperatures close to the critical temperature of carbon dioxide (31°C).

Few experimental data on the solubility and extractability of alkaloids such as emetine, cephaeline, reserpine, vincristine (4), caffeine, theophylline, theobromine (6), codeine, thebaine, papaverine (7), quinine, morphine (8), pyrrolizidine (9), trigonelline (10), and ephedrine (11) in various supercritical solvents such as carbon dioxide, ethylene, nitrous oxide, and fluoroform have been obtained over the past few years. While there is a growing market for decaffeinated products (12), extraction of the many alkaloids encountered in natural plants with supercritical carbon dioxide is yet to reach a desirable level of industrial application (5).

In this chapter, we present information on the potential application of this promising technology: extraction using supercritical fluids in the isolation and recovery of alkaloids from natural products. This presentation includes a brief summary of the physiological effects and applications of alkaloids, a description of commercial decaffeination applications of coffee and black tea, followed by experimental laboratory data on the potential use of carbon dioxide for the isolation of a number of alkaloids from natural products, and future prospects in the isolation of important alkaloids.

II. PHYSIOLOGICAL EFFECTS AND APPLICATIONS OF PURINE ALKALOIDS

The principal purine alkaloids—caffeine, theobromine, and theophylline—are consumed in large scale through the daily ingestion of coffee, tea, herbal maté, cola drinks, and chocolate as well as in analgesics, diuretics, and other pharmaceutical products. Physiological effects of methylxanthines on humans have received substantial attention, due largely to the high coffee consumption worldwide. All three purine alkaloids do, however, exhibit stimulant and psychomotor effects, with caffeine and theophylline having highest activity. Theophylline is also known to exhibit more diuretic effects as it inhibits the tubular reabsorption of water and sodium in kidneys (13). It is also used as a vascular as well as a bronchial dilator (14). Theobromine is found to have toxic effects on virtually all animals tested, including chickens (15), dogs (16), mice (17), rats (18), rabbits (19), and humans (20). Recent interest in caffeine and theobromine is also motivated principally by their reproductive toxicities and their potential to induce fetal malformation through effects on embryo development. Furthermore, teratogen effects in animals are also known to occur as a result of biotransformation when consuming products containing caffeine, theophylline, and theobromine (21). Finally, it is important to note that the presence of these alkaloids in products such as cocoa beans could prove to be a limiting and restrictive factor in its potential as a nourishing food in developing countries (22).

III. COMMERCIAL SCALE APPLICATIONS

Large commercial scale operations for the extraction and isolation of valuable alkaloids from natural plants for the pharmaceutical and cosmetic industries have been developed and operated for many years (23–25). Among the best known operations using supercritical carbon dioxide are the decaffeination processes of coffee and black tea and the removal of nicotine from tobacco. With the increase of consumption of decaffeinated coffee worldwide, caffeine is now a major byproduct of coffee decaffeination with significant contribution in plant amortization (26–29). There are several patents and commercial processes for the decaffeination of coffee beans (30–32) and black tea (33, 34). These processes employ a number of organic, nonorganic, and supercritical solvents. Except when supercritical carbon dioxide is used, the extraction process either is nonselective for caffeine or brings with it the potential risk of toxic residues in the decaffeinated products that could prove to be harmful to the consumer.

Extraction with supercritical carbon dioxide as a substitute for chemical solvents eliminates the risks of potential toxic residues due to the inert nature of CO_2 (35, 36) and thermal degradation of the decaffeinated product due to the mild operating temperatures (near the critical temperature of CO_2 of 31°C), besides being highly selective for caffeine. These characteristics result in the preservation of most of the qualities of the original non-decaffeinated product.

In what follows, a more detailed description of decaffeination operations of coffee beans and black tea using conventional and supercritical fluid solvents is presented.

A. Decaffeination of Coffee Beans

Coffee, one of the most widely known natural products, contains 0.8–2.5% weight of caffeine, depending on the species (*Coffea arabica, C. canephora, C. liberica, C. racemosa*, etc.). It is used as a daily beverage in many parts of the world. Green coffee beans are cultivated mainly in South America and Africa.

1. Decaffeination Using Conventional Solvents

Commercial coffee decaffeination is carried out on green coffee beans before roasting in order to minimize loss of flavor and aroma. These processes involve the swelling of raw beans with water, caffeine removal with water-insoluble organic solvents, steam stripping or distillation to remove residual solvent from extracted beans, and drying of decaffeinated coffee beans to their initial humidity.

Organic solvents commonly used include toxic and flammable substances such as benzene, incombustible and highly volatile chlorinated methylene chloride (37), ethyl acetate (38), methyl acetate, ethylmethylketone, and trichloroethane (39, 40). Water (40–42) is an ideal solvent for decaffeination except for its high nonselectivity for caffeine, which results in the extraction of other water-soluble substances. Caffeine is subsequently recovered by washing the aqueous extracted solutions with organic immiscible solvents such as methyl chloride or adsorption of caffeine on activated carbon. As noted earlier, these conventional processes suffer from two serious limitations: the risk of toxic residues in the decaffeinated products when using organic solvents and/or the nonselective extraction when using water, which results in the removal of important constituents together with caffeine from the original products.

2. Decaffeination Using Supercritical Carbon Dioxide

Supercritical carbon dioxide (SC-CO_2) is an excellent and selective solvent for caffeine as it solubilizes caffeine and does not remove other important water-soluble components from coffee beans. The commercial decaffeination of coffee is the first important industrial application of supercritical extraction in the food industry with plants constructed and operated in Germany and in the United States (Table 1). The plant owned and operated by General Foods has a yearly production capacity of 50×10^6 kg of coffee beans (43). The German plant processes nearly 30×10^6 kg of green coffee beans per year (44). In this decaffeination process, color and odor of the extracted beans are totally preserved, which is not always achieved when using conventional extraction processes (45). Extracted caffeine is reported to be of a very high purity and is easily isolated and refined to provide a salable product (45).

Zosel's patents on the decaffeination of green coffee beans using SC-CO_2 (30, 31, 46, 47) served as the basis for the development of these commercial

Table 1 Commercial Plants Using SC- CO_2 Extraction

Process/alkaloid extraction	Manufacturer
Coffee decaffeination	Kaffee HAG AG, Bremen, Germany
	General Foods, Texas, USA
	Hermsen, Bremen, Germany
	SKW-Trostberg, Poszzillo, Italy
Tea decaffeination	SKW-Trostberg, Munchmuenster, Germany
Nicotine extraction	Philip Morris, Virginia, USA

Source: Data from Ref. 23.

decaffeination plants. These patents propose, basically, three possible processes. In the first process, moistened green coffee beans placed in the extractor are contacted with CO_2 at 16–22 MPa and 70–90°C, where caffeine diffuses from the beans into the CO_2 stream. This caffeine-saturated stream is passed into a water-washing tower operated at 70–90°C where caffeine is retained and the water-saturated CO_2 is recycled to the extractor. Caffeine retained in the wash water is separated by distillation. Decaffeinated coffee beans have a residual caffeine content of only 0.02%.

In the second process, caffeine is extracted from coffee beans under the same conditions as described in the first process while the caffeine-saturated CO_2 stream is passed through a bed of activated carbon where caffeine is retained. Adsorbed caffeine is subsequently recovered from activated carbon.

In a third alternative presented in these patents, a mixture of moistened green coffee beans and activated carbon pellets is charged into the extractor (1 kg of activated carbon/3 kg of coffee beans). CO_2 is subsequently introduced into the extractor and maintained at 22 MPa and 90°C. Caffeine diffuses from coffee beans into carbon dioxide until reaching the activated carbon. After the extraction, coffee beans are separated from the activated carbon pellets using vibrating sieves.

Modifications of Zosel's processes as well as new decaffeination processes were also suggested in U.S. and German patents as reported by Lack and Seidlitz (12) and McHugh and Krukonis (48). These new processes and modifications involve (12, 48, 49) the following: (a) incorporation of two smaller pressure vessels that are periodically charged and discharged with coffee beans allowing a continuous extraction and using less quantity of CO_2 than a discontinuous process; (b) recovery of extracted caffeine by washing the CO_2 stream with water and subsequent evaporation or crystallization; (c) use of other solvents such as NO_2, NH_3, and CHF_3 for caffeine extraction; (d) use of an ion exchange resin with temperature change to retain caffeine instead of activated carbon; (e) use of liquid CO_2 as a solvent; (f) addition of cosolvents such as acetone, methanol, and ethanol to increase solubility of caffeine in SC-CO_2 and decrease the operating pressures. Other proposed modifications included the use of mixed solvents such as carbon dioxide and propane, butane, or ethane (48), and the utilization of roasted coffee as described in the patents of Vitzthum and Hubert (32, 50) who employed a multistage process in which flavor and aroma were first extracted in the form of coffee oil. The oil-free coffee was subsequently moistened and decaffeinated using a stream of water-saturated SC-CO_2. The decaffeinated roasted coffee is then spray dried and aromatized with the coffee oil extracted earlier.

Pilot plants in operation using the methods proposed by Zosel are also described in the literature (12, 45). These commercial and pilot plants consist, basically, of extractors, pumps, washing columns, and heat exchangers.

An interesting economic projection of a supercritical plant with daily feed rates of 32,000 and 64,000 kg of green coffee containing 12% of moisture operated during 330 days was presented (51). Decaffeinated whole green coffee beans with 3% of the original caffeine content and an aqueous caffeine solution were the main products. The extraction conditions considered were 14–35 MPa and 70–130°C and the separation conditions of 5–10 MPa and 15–50°C. Processing costs were estimated by considering (a) electric power, steam, cooling and process water, and carbon dioxide; (b) operation labor and supervision; and (c) maintenance, taxes, insurance, and plant overhead. Estimated costs were found to be $0.83 and $0.68 per kg of coffee produced for plant capacities of 32,000 and 64,000 kg, respectively.

3. Decaffeination with SC-CO_2 and Conventional Solvents: Main Advantages and Disadvantages

Economical studies of Lack and Seidlitz (12) have shown that although the initial investment cost of the SC-CO_2 plant is higher, this process provides a higher profit per ton of coffee processed than does the ethyl acetate process, due to the excellent quality of both decaffeinated coffee and caffeine produced and negligible losses in the SC-CO_2 process. Carbon dioxide extracts caffeine without affecting the reduced sugar and amino acid contents. These compounds need to be preserved because they are converted to flavor and aroma during the roasting process (45). The flavor and appearance of decaffeinated coffee beans obtained with the carbon dioxide process are very close to those in the original un-decaffeinated coffee and superior to the product obtained from other conventional decaffeination methods. This process is recognized by the consumer as a "natural" decaffeination technique for the high qualities of both products and byproducts. The total quantity of caffeine recovered is much higher using compressed carbon dioxide than ethyl acetate. Supercritical carbon dioxide decaffeination is also considered a clean and environmentally acceptable technology.

Steam cost is another factor that contributes to the higher process cost when using ethyl acetate. For the pretreatment of beans, both processes require steam to swell the beans but only the ethyl acetate process requires almost four times more steam to strip residual solvent from the final product.

The limiting factor for the use of the CO_2 process is, however, the capital cost due mainly to the highly specialized equipment needed for a safe plant operation. Necessary equipment includes compressors for recirculation of CO_2, separators and extractors with baskets that support high pressure and allow fast operation, adequate piping, as well as safety and control systems to avoid interruptions in production and precaution against explosion.

B. From Black Tea (*Camellia sinensis*)

Tea, a natural product, contains approximately 2.0–3.5% caffeine (52). The most common types of tea are green and black. It is used as a daily beverage worldwide, mainly in China and India. Most decaffeinated tea is produced and consumed in North America and Europe.

1. Tea Decaffeination Using Conventional and $SCCO_2$ Processes

These processes are similar to those used for coffee decaffeination and normally employ organic solvents, water, or supercritical fluids. Organic solvents such as methylene chloride and ethyl acetate are employed in a similar manner to that used in the decaffeination of coffee beans (45). As pointed out earlier, the operations using organic solvents bring with them the risk of leaving toxic residues in decaffeinated products, whereas the loss of valuable water-soluble tea constituents is markedly increased when using water as the solvent due to its nonselectivity for caffeine. Extraction with supercritical carbon dioxide is selective for caffeine and eliminates the risk of possible toxic residues in the decaffeinated products. Vitzthum and Hubert (34) describe a multistage procedure that avoids the loss of flavor and aroma when decaffeinating black tea. Aroma components are removed from tea by extraction with dry SC-CO_2 at 25 MPa and 50°C. The dearomatized leaves are subsequently moistened and decaffeinated with water-saturated CO_2. The decaffeinated leaves are vacuum dried at 50°C and rearomatized by contact with the expanded CO_2 solution containing the aroma components. This procedure is also used for the production of caffeine-free instant tea. In this case the decaffeinated tea is extracted with hot water and the extract is freeze dried. The powder produced is impregnated with tea aromas using a solvent that is subsequently evaporated.

An SC-CO_2 tea decaffeination plant is currently operated by SKW/Trostberg in Meunschmeunster, Germany (53). Decaffeinated ice tea with tropical flavors, where decaffeination is obtained using SC-CO_2, was reported to have been introduced into the market by the Hansen Company (54).

From a laboratory study, Klima et al. (33) reported the extraction of black tea using wet carbon dioxide at 25.5–35 MPa and 50–80°C. The moistened tea with 15–50 wt% water content is packed into the extractor in alternate layers with activated carbon. Before leaving the extractor, the CO_2 stream passes through the bed of pure adsorbent placed inside of gas-permeable bags or tubes where extracted caffeine is adsorbed.

C. Potential Applications: Extraction of Methylxanthines from Natural Products

The potential extraction of methylxanthines—caffeine, theophylline, and theobromine—a major group of purine alkaloids, from coffee beans (55–57), cocoa nibs (58), and shells (59) and guaraná seeds (60) has also been investigated over the past few years. In what follows, we present a brief review of laboratory experiments and results obtained.

1. From Maté Tea (*Ilex paraguariensis*) Leaves

Maté tea is a beverage prepared by the infusion of dry maté leaves and is traditionally consumed in southern Brazil, Argentine, Paraguay, and Uruguay (61, 62). A careful inspection of the chemical constituents of *Ilex* species (Table 2) reveals the reason for current and successful use of this natural product as a stimulant, an antirheumatic, and a diuretic. Nevertheless, a high intake of herbal maté tea could provoke irritability and insomnia, and the possibility of developing cerebral depression, nervous tremor, and numbness. Several laboratory studies (63–66), on the other hand, have shown that polyphenols found in this tea could inhibit the formation and growth of tumors.

Similar to the decaffeination of coffee beans, removal of caffeine from maté tea leaves can be performed using organic solvents or water. While there are problems associated with the use of chemical solvents (potential toxic residues in extracted products) and water (nonselectivity, which results in loss of valuable flavor components) (68, 69), the use of carbon dioxide at supercritical conditions proved to be potentially convenient in the extraction of methylxanthines from natural products (44, 68).

While there are many patents for the use of SC-CO_2 as a solvent to extract caffeine from coffee beans and *Camellia sinensis* tea leaves (44, 56, 59), little is known about the extraction of methylxanthines from maté tea.

During the past 2 years, our group has conducted an investigation on the extractability of methylxanthines from *Ilex paraguariensis* (70) where we used a semicontinuous-flow, high-pressure system purchased from Autoclave Engineers (Erie, PA). The major components of the apparatus included positive liquid displacement pumps for solvent delivery, high-pressure extraction vessels,

Table 2 Taxonomy, Chemical Compoisition, and Other Characteristics of *Ilex paraguariensis*

Taxonomy	Other characteristics
Division: Anthophyta	Consumed part: leaves and some barks
Type/Subtype: Magnoliopsida/Rosidae	Color: green
Order: Celastrales	Taste: astringent and sour
Family: Aquifoliaceae	
Gendus: *Ilex*	Humidity
Specie: *paraguariensis, paraguayiensis*	- "in natura": 50–60 wt%
Common names: yerba maté, maté, erva maté, Paraguay Cayi, Paraguai Tea, South American Holly	- commercial: 8–10 wt%
Chemical composition/quantity (100 g)	**Alkaloids (mg/kg)**
Protein: 10.89	Theophylline: 142 ± 6,[a] 768 ± 3[b]
Carbohydrate: 12.04	Theobromine: 340 ± 7,[a] 209 ± 5[b]
Starch: 4.55	Caffeine: 5371 ± 161,[a] 8375 ± 251[b]
Glucose: 3.84	
Fiber: 16.96	

[a]Old leaves
[b]New leaves from branches with fruit
Source: Data from Refs. 61 and 67.

and three separator flasks in series (Fig. 1). Flow rates and accumulated gas volumes passing through the apparatus were controlled with micrometering valves and measured with a flow computer-measuring device. Heating tapes were used to maintain constant temperature in the extraction section and in valves to prevent freezing of solvents or solid solute precipitation following depressurization. Pressure in both extractors was monitored with a digital transducer system. Maté leaves were extracted with liquid CO_2 at 25.5 MPa and 70°C; samples were collected and analyzed for purine alkaloids.

A supercritical extraction curve for maté tea revealed that high caffeine removal rates were obtained in the early stages of the extraction with extraction rates diminishing at later stages (Fig. 2). A similar qualitative behavior was observed for theophylline and theobromine extraction (Fig. 3). These results reveal the higher selectivities of CO_2 for caffeine followed by theobromine and theophylline. The cumulative amounts of methylxanthines extracted from maté tea amounted to 4308, 348, and 47 mg of caffeine, theobromine, and theophylline per kg of dry maté tea, respectively. Considering that different maté leaves were used, these values are in good agreement with alkaloid contents in maté tea reported by Mazzafera (67).

After about 7 h of extraction, 94%, 68%, and 57% of extracted caffeine,

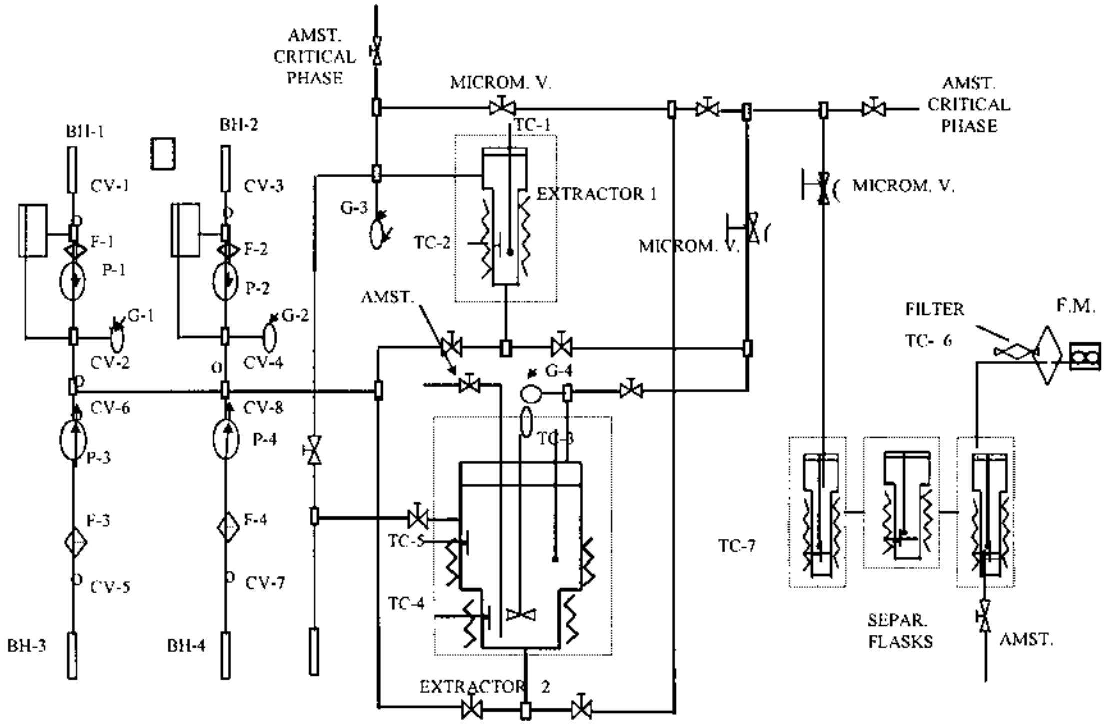

Figure 1 Experimental apparatus. BH(1-2), solvent; BH(3-4), cosolvent; G(1-2-3-4), pressure indicator; P(1-2-3-4), pumps; F(1-2-3-4), filters; V. microm., micrometrical valve; CV(1-2-3-4-5-6-7-8), valves; TC(1-2-3-4-5-6-7), thermocouples; Extractor 2, extractor with stirring and a window; Separ., separator flasks; Amst., sample; FM, flow measurement (68, 70).

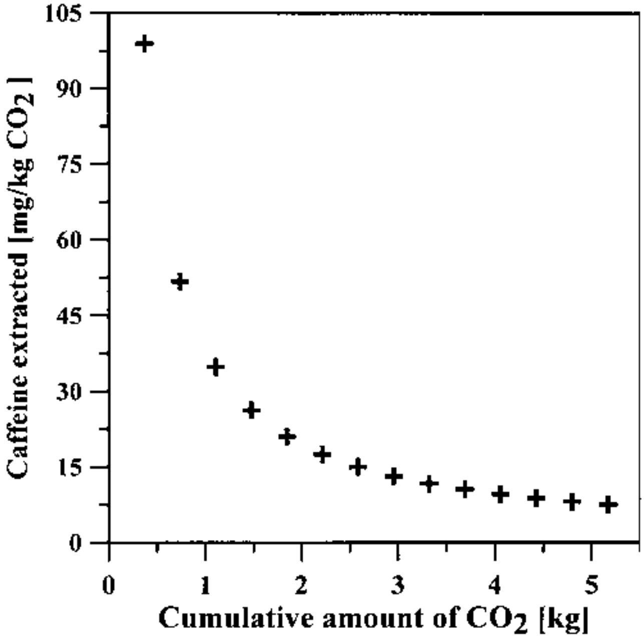

Figure 2 Caffeine extraction curve for the fractionation of maté tea at 25.5 MPa, 70°C, and a CO_2 flow of 0.9–1.2 g/min.

theobromine, and theophylline in the plant matrix, respectively, had been recovered. By the last fractions, 99.9% of caffeine, 96% of theobromine, and 95% of theophylline had been removed. Fractions obtained at the late stages became each time richer in theobromine and theophylline and could provide an interesting approach to the separation of extractable methylxanthines into fractions of varying concentrations.

2. From Cocoa (*Theobroma cacao*) Beans

Cocoa beans are the seeds of the tropical cocoa tree, *Theobroma cacao*. These beans are used as raw material for producing chocolate and cocoa powder, which are subsequently used in the manufacturing of other products such as cake fillings, pudding powders, ice cream, and cocoa beverages (chocolate), among others.

A theobromine content of about 1–2% and a caffeine content of 0.1% of dry nut are reportedly found in the species *Theobroma cacao* (71, 72). During fermentation, theobromine and caffeine migrate to the shell. Theobromine contents of about 2% of the dry shell were reported (73). The shells represent about

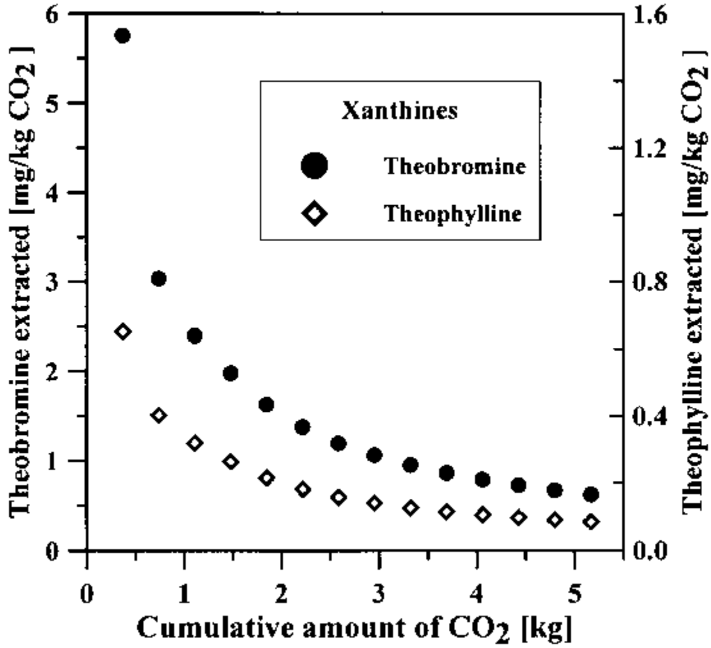

Figure 3 Dimethylxanthine extraction curves for fractionation of maté tea at 25.5 MPa, 70°C, and a CO_2 flow of 0.9–1.2 g/min.

10–12% of the nut's weight. Once theobromine is extracted, shells are nowadays used as animal ration. For example, considering the production of 150×10^6 kg of cocoa nuts [Brazilian production 1980–1981 according to CEPLAC (74)] results in 13×10^6 kg of shells and potentially 30,000 kg of theobromine available for use in food, pharmaceutical, and cosmetic industries (72).

Cocoa beans are also a potential source of high-quality cocoa butter (Table 3) that is used in a variety of applications in the cosmetic and pharmaceutical industries. Furthermore, the residue material obtained in cocoa bean processing is fermented to produce organic fertilizers.

Cocoa butter with the characteristic and pleasant flavor and odor of cocoa beans contains many different types of triacylglycerols. Hexane is one of the conventional solvents used for the extraction of cocoa butter. Due to the toxic nature of hexane, a maximal residue of 5 mg/kg is allowed in the final cocoa butter.

Methylxanthines could be extracted from cocoa beans conventionally in a four-step process (75): extraction with warm water, purification using an adsorbent, recovery of the xanthines from the adsorbent, and, finally, addition of

Table 3 Composition of Cocoa Beans and Cocoa Shells

Component	Cocoa bean (wt%)	Cocoa shell (wt%)
Moisture	5	4.5
Fat	54	1.5
Theobromine	1.2	1.4
Caffeine	0.2	—
Polyhydroxyphenols	6	—
Crude protein	11.5	10.9
Mono- and oligosacharides	1	0.1
Starch	6	—
Pentosans	1.5	7
Cellulose	9	26.5
Carboxylic acids	1.5	—
Other compounds	0.5	—
Ash	2.6	8

Source: Data from Refs. 71 and 72.

residual concentrated aqueous extract to the cocoa beans to reestablish the solid content of the bean.

Extraction of theobromine and caffeine from cocoa seeds (58, 76) and shells (59) can be also done using supercritical carbon dioxide. Margolis et al. (77) described a process in which stimulants are removed from cocoa by SC-CO_2 extraction of water-swollen nibs at 30 MPa and 90°C. It was earlier indicated that water acted as a chemical agent that freed caffeine linked to coffee substrate allowing the caffeine to be extracted by SC-CO_2 (56). As caffeine is similar to theobromine, one could assume that the same effect would be observed in the supercritical extraction of theobromine from cocoa beans.

A characterization of cocoa extracts obtained with SC-CO_2 as a function of temperature and pressure was presented by Rossi et al. (78). This work pointed to improved extraction yields at pressures exceeding 40 MPa but resulted in a nonselective process. For a better extraction of all cocoa fat, fine milling of the lipid bearing material was required. Experimental data on the extraction of cocoa butter from three substrates—nibs, shells, and liquor in which approximately 20%, 60%, and 100%, respectively, was obtained at 40 MPa and 80°C in 5 h—were also presented (79).

Li and Hartland (58) used ethanol as a cosolvent for the SC-CO_2 extraction of methylxanthines from cocoa nibs. The solubility of theobromine increased with ethanol concentration in the supercritical solvent. However, extraction with ethanol as a co-solvent proved to be more selective for cocoa butter than for theobromine, contrary to what was observed when using an aqueous cosolvent (77). Brunner (59) investigated the influence of water content on the extraction of theobromine from cocoa seed shells using supercritical carbon dioxide at 30 MPa and 80°C and reported that the solubility of theobromine in water-saturated $SCCO_2$ was found to be much less than that of caffeine. The extraction yield was also found to increase with pressure and moisture content. Furthermore, higher extraction temperatures favored the removal of xanthines and cocoa butter, whereas grinding and swelling of the starting material with water did not improve the extraction (80).

Recently our group explored the extractability of theobromine from Brazilian cocoa beans (81). Results revealed the extraction of almost 89% of the original theobromine at 20 MPa and 70°C using 10% ethanol as a cosolvent of supercritical carbon dioxide. When extraction was performed with dry or water-saturated carbon dioxide or at lower pressures or temperatures, additional time and larger amounts of carbon dioxide were needed to achieve the same yield. Furthermore, the extraction was coupled to an adsorption step using activated carbon where extracted theobromine and cocoa butter were simultaneously adsorbed and separated from the CO_2 solvent.

3. From Guaraná (*Paullinia cupuana*) Seeds

Guaraná is a bush plant native to Brazil, with the seeds being the only part used for human consumption. The seeds are commonly used in concentrated and soft drinks and as ingredients of a variety of pharmaceutical products. They are the richest source of caffeine, with 3–6% weight on a dry basis (68), and have shown aphrodisiac and stimulant effects, acting on the cardiovascular and nervous systems and kidneys. These effects are attributed to the rich caffeine content encountered in guaraná seeds (82, 83).

South American Indians have traditionally toasted, ground, and mixed the seeds with water to form a paste that can be molded into different configurations (rods and sticks) to be used in special rituals. The toasted seeds can be ground to a powder and added to syrups as an essence or to water to form consumable drinks.

Similar to coffee and tea decaffeination, caffeine removal of guaraná seeds could be performed using organic solvents such as dimethyl chloride and water (84). The use of supercritical carbon dioxide eliminates the risks of toxic residues in the extracted products and the long nonselective extraction presented with water as a solvent. As observed with coffee beans, water can act as a valuable cosolvent leading to a substantially improved extraction yield (56, 60, 68).

The literature contains the limited data of Mehr et al. (60) who reported extractions for pressures of 13.74–27.47 MPa and temperatures of 35°C, 45°C, and 55°C. Our recent findings (85) revealed some interesting information on the ability of supercritical fluids in the decaffeination of widely consumed caffeine-rich natural guaraná seeds with water-saturated supercritical carbon dioxide. The extraction was performed using a semicontinuous-flow high-pressure microextraction apparatus at 40°C and 70°C and pressures of 10, 20 and 40 MPa. Carbon dioxide flow rates of 3 and 5 L/min were used.

Extraction curves showed the existence of a thermodynamic solubility–dependent, an intermediate, and a diffusion controlled regions. Extraction at 40 MPa and 70°C using water-saturated supercritical carbon dioxide at a flow rate of 3 L/min allowed the removal of almost 99% of initial caffeine content in wet ground guaraná seeds in 240 min. When extractions were performed at lower pressures or temperatures, additional time and larger amounts of carbon dioxide were needed to achieve the same yield. Increasing the carbon dioxide flow rate did not present any economic advantages unless the extraction was limited to the thermodynamic solubility region. For total extraction of caffeine, the use of low flow rates resulted in similar final product yield but at lower solvent consumption. A retrograde behavior for the extraction of caffeine from guaraná seeds was also observed at 10 MPa for the 40–70°C isotherms.

4. From Other Natural Matrices

Many alkaloids encountered in natural plant species are of interest as anticancer agents. Supercritical carbon dioxide extraction has provided a solution for the extraction of active components from some natural matrices without the risk of degradation or contamination with toxic solvents. Besides the applications already cited, the extraction and isolation of chemotherapeutic pyrrolizidine alkaloids (monocrotaline) from the seeds of *Crotalaria spectabilis* using supercritical carbon dioxide with ethanol and water as cosolvents has also been reported (9). In this operation, 94–100% of the monocrotaline was obtained using an ion exchange resin column, demonstrating the potential and effective extraction and isolation of other alkaloids of this class. Nicotine and nornicotines are alkaloids encountered in *Nicotiana* (86, 87) and are used as a powerful insecticide as well as in epidermal patches that help ease the difficulties associated with cigarette addiction. Nicotine has been extracted from tobacco using carbon dioxide–water mixtures (88, 89), and nowadays there is even an industrial plant for its extraction operating in the United States.

Supercritical carbon dioxide has also been employed for the extraction of alkaloids such as quinine and morphine (8). Morphine is one of the major alkaloids isolated from plants by conventional methods for industrial use. Annually, approximately 160,000 kg of morphine (24) is purified and 90–95% of that is methylated to codeine (25), which is then either used directly or chemically converted to a variety of derivatives that find use as analgesics. The illicit production of morphine for acetylation to heroin reaches almost 10 times that amount, totaling more than 1.2×10^6 kg/a (90).

Few alkaloids were also extracted with other supercritical fluids such as fluoroform (CHF_3), which is an attractive solvent with a good polarity (1.6D) and accessible critical parameters. The solubilities of opium alkaloids—codeine, thebaine, papaverine, and noscapine—in supercritical CO_2, N_2O, and CHF_3 have been studied by Stahl et al. (7, 91) at 15 MPa and 40°C. With the exception of codeine, all other alkaloids tested exhibited higher solubility in CHF_3 than N_2O and CO_2. Sanders (39) has also presented some production of alkaloid extracts from vegetable matter using supercritical carbon dioxide.

D. Future Prospects

During the past 20 years, the use of supercritical fluid solvent technology has advanced rapidly. Supercritical fluids have been applied to areas as diverse as food industry, pharmaceutical, polymer, oils, petroleum, textile, biotechnology, among others.

Some alkaloids that offer future prospective for pharmaceutical applications include emetine, an active ingredient of Ipeca (*Cephaelis ipecacuanha* Rubiaceae) used by South American Indians for the treatment of amoebic dysentery and other rubiaceous species. Quinine, medically used as in the treatment of malaria, and quinidine, used as an antiarrhythmia drug (92), are also alkaloids that could potentially be extracted from natural plants like *Cinchona* shells. Physostigmine, also called eserine, is another alkaloid that could be extracted from the calabar bean of West Africa (*Physostigma venenosum Balf)* and used clinically in the management of glaucoma by reduction of intraocular tension (93). Other potential alkaloids include colchicine, an ancient and well-known drug used for the management of gout (94), from the plant *Colchicum autumnale*, the autumn crocus or meadow saffron, and the glory lily *Gloriosa superba*; bark extracts from the Yuzuriha tree (*Daphniphyllum macropodum*), which have been used for centuries as a folk remedy for asthma (95), and the tropane alkaloids in solanaceous plants that have been traditionally used for their medicinal, hallucinogenic, and poisonous properties (96, 97). The narcotic, anesthetic, and psychostimulant cocaine (98) is a tropane alkaloid found outside of the Solanaceae in *Erythroxylum coca* (Erythroxylaceae). The initial source of taxol was the bark of *Taxus brevifolia*, which proved to be efficient for refractory ovarian cancer in 1992 and since 1994 being tested for breast cancer and other tumors (99).

Current efforts to apply metabolic engineering to increase the production of economically important alkaloids continue and should expand to include more plant species and compounds. The enormous worldwide effort to screen plants for new biologically active compounds is expected to bring new drugs, some of which will probably be alkaloids, to the market. In the coming years, considerable interest may thus be expected in the field of plant cell biotechnology and metabolic engineering of secondary metabolism by the pharmaceutical industry.

Recent data obtained by this research group at the State University of Campinas (UNICAMP) in Brazil, in collaboration with Professor Brunner's group at the Technical University of Hamburgh-Harburg (TUHH) in Germany, have revealed interesting and very useful information on the extraction of methylxanthines from natural products such as guaraná seeds, maté leaves, and cocoa beans using supercritical carbon dioxide and ethanol, and on the extraction of caffeine and theobromine along with cocoa butter from Brazilian cocoa beans (85, 100, 101). These findings point to new opportunities in the extraction of other important active principles in natural plants of interest to pharmaceutical, food, and cosmetic industries.

For research on alkaloids, the future looks very exciting leading to a number of interesting applications in the production of specialty chemicals and pharmaceuticals (92). Environmental and ecological factors can have a considerable impact on future processes.

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