OIL OF CATNIP BY SUPERCRITICAL FLUID EXTRACTION

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ABSTRACT

Supercritical fluids and in particular supercritical fluid carbon dioxide have shown to be a viable technology for a variety of extractions. While process conditions require high pressures, technological advances have now made it feasible to acquire such equipment for the laboratory. Traditionally, the oil of catnip has been isolated by steam distillation. Working with dried plant material, we have introduced supercritical fluid carbon dioxide as an alternative extraction medium. Results of our work including characterization of the major constituent components will be presented.

INTRODUCTION

A supercritical fluid (SF) is created by heating any substance above its critical temperature and raising its pressure above its critical limit as well. Critical temperature refers to the highest temperature at which a gas can be converted to a liquid through an increase in pressure. Similarly, critical pressure is the highest pressure a liquid can be converted to a gas by an increase in temperature. Parameters such as the density, diffusivity and viscosity of SF's are therefore intermediary of liquids and gases. While the solvent properties are representative of that of a liquid state, the diffusivity and viscosity are characteristic of a gas. Maintaining a constant density and increasing temperature will increase the diffusivity of the SF while simultaneously decreasing its viscosity. If temperature is held constant, an increase in pressure will oppositely affect the SF.

Carbon Dioxide (CO_2) is environmentally compatible, available at high purity, has an easily attainable critical point (31.0°C and 1070 psi), low combustibility and low toxicity. Additionally, CO_2 possesses a high quadrapole moment and lacks a dipole moment, allowing for the dissolution of both polar and non-polar solutes.

Supercritical fluid extraction is essentially a typical solvent extraction with the modification that the SF solvent undergoes a change of state prior to the extraction process and changes state again afterwards to remove itself from the analyte. Specifically, the substance chosen as SF solvent is cooled from its gaseous state into a liquid, then pressurized above its critical pressure threshold so that it is essentially a high-pressure liquid. It is then heated above the critical temperature to become supercritical. The SF then flows into a vessel of known volume containing the solute. The SF is allowed to "soak" into the solute for a predetermined amount of time known as the static phase of the extraction, then continuously cycled through the vessel in dynamic phase. The SF is then completely removed from the vessel through depressurization of the vessel via a restrictor in order to cause the SF to decrease in density and return to a gaseous state. The analyte is deposited in a collection vessel while the SF effectively eliminates itself (Taylor, 1996).

In this experiment we study the efficiency of supercritical fluid extraction of oil of catnip using CO_2 as compared to traditional solvent extractions using significant volumes of diethyl ether.

A common conventional method used to extract the essential oil from catnip is steam distillation. Chopping up the plant stems, leaves, and flowers and placing them in a pressure cooker for approximately one hour will accomplish this. Using this method produces, on average, 0.3% the plant's weight in volatile oil. Early work by McElvain *et. al* (1941) found that the oil consists of three fractions: nepetalactone (40-50%), nepetalic acid (33%), and a neutral fraction (14%).

Catnip (*Nepatia cataria*) is a perennial herb plant naturalized in North America and native to parts of Eurasia. The species name *cataria* is from the Latin word for cat which may indicate that there was an early recognition of a cat's attraction to the intoxicating substance. The fresh leaves of this relative of the mint family have a minty aroma which gives rise to the another common name for the plant, catmint (Waller *et al*, 1969). Catnip plants can reach a height of one meter and will usually display spike like purple-spotted white flowers. They flourish in temperatures ranging from 7 to 9°C with well-drained soils. Moderately acidic (4.9) to neutral (7.5) soils ensure the best success rates for growing these plants. While commonly considered a weed in the gardens of northeastern United States, bees thrive on the flowering tops of the plant. These tops are also dried in order to preserve the color and fragrance. The entire plant is utilized when the catnip is harvested for cats. It is not toxic to pets.

Nepetalactone is the volatile oil produced from this plant. This is what actually gives the plant its distinct odor. Veterinarians believe it to be similar to the hormone naturally produced by female cats when they need to find a mate. This may be why about 50% of cats at the reproductive age respond to the toys stuffed with catnip and the kittens and very old cats never do. The behavioral responses exhibited after exposure to catnip is a hybrid of responses associated with their predatory behavior and mate luring behavior. These behaviors include rolling on their backs, rhythmically kicking back feet, pawing and marking, becoming extra playful, begging to be petted, and purring more (Harney *et al*, 1978).

The vomeronasel organ located above the palate is the receptor site for nepetalactone. While this organ may not be unique to the feline genus, the receptor site for nepetalactone is. Many animals use this organ to gain direct and specific contact with the chemical cues released in their environment. These cues aid in social interactions such as sexual relationships. Since it has to reach this site above the palate, catnip is only effective after inhalation.

Catnip has been used for ornamental and culinary purposes as well as a domestic folk-medicine remedy. The leaves and shoots have been used in sauces, soups, and stews. Leaves and flowers are usually steeped to make a calmative herbal tea used to hasten slumber and to achieve a restful night's sleep. Medicinally, catnip has been used as an antispasmodic, diaphoretic, emmenagogue, nervine, stimulant, and mild sedative. It can also be used to treat diarrhea, colic, the common cold, cancer, tension, and anxiety. Extract of catnip is reported to exhibit juvenile hormone activity. Recently, it has been found to repel cockroaches one hundred times better than a powerful insect repellent. The rare more potent form of nepetalactone with a slightly different atomic structure killed flies. (DoVing and Trotier, 1998).

METHODS

Conventional Approach

The oil of catnip was first extracted using the polar solvent diethyl ether. Crushed catnip stems and leaves weighing 10g were placed in a 1000 mL separatory funnel. The oil was extracted using three washes of 125mL diethyl ether each. The solvent/extract solution was placed in preweighed round bottom flasks in order to concentrate the oil by rotary evaporation. The average mass of oil obtained was 1.12g per 10g of crushed catnip. The total mass of oil collected from the 30g of crushed dried catnip was 3.35g. The concentrated extract sample was purified by column chromatography using a 100g column. The solvent system was volume/volume mixtures of ethyl acetate (0-9%) in petroleum ether. Seven distinct groups of collected fractions were identified by thin layer chromatography. These groups were further analyzed using proton nuclear magnetic resonance spectroscopy. The fractions with a TLC R_f value of 0.25 and 0.29 in 5% ethyl acetate/petroleum ether were determined to be a mixture of nepetalactone isomers.

Supercritical Approach

Oil of catnip was extracted via the supercritical fluid carbon dioxide in an SFT1000 Supercritical Fluid Processing Unit (SFPU). The dried catnip (consisting of leaves, stems, and buds) was crushed and 17.533g were packed into a 50mL stainless steel vessel. After securing the vessel into place, the SFPU was set to the appropriate conditions. The conditions were checked using the “system status” function, and conditions were altered using the “rest parameter editor” function. The initial temperature for the SFE/SFR was set to 40°C while the initial pressure setpoint was 2000 psi. During this time, the vessel filled with liquid carbon dioxide. The pressure was raised in increments of 2000 psi by use of a pneumatic pump until the desired pressure of 6000 psi was obtained. The SFPU operated in the static mode for 30 minutes allowing the carbon dioxide time to soak through the catnip. After this time, the unit was set to dynamic mode for 7 minutes, and the restrictor valve was opened manually 13 full turns (completely opened is 17 turns) in the counter clockwise direction. It was important to monitor the restrictor valve temperature at this time using the “system status” function. If the temperature, set to approximately 60°C, fell below 5°C, the restrictor valve had to be turned in the clockwise direction to maintain the temperature above the freezing point. During this time, supercritical carbon dioxide extracted the oil from the sample and carried it to the collection jar. After the completion of the 7 minutes, the pressure setpoint was reset to zero using the “rest parameter editor.” (The mode was still dynamic). The restrictor valve was turned in the counter clockwise direction allowing a rapid fall in pressure. When the pressure fell below 1200 psi, the “open depressurization valve” function was selected to provide the final rapid depressurization.

The weight of the catnip oil extracted was 0.890g. The unit was flushed with ethyl acetate to clean out any remnants. The additional oil was collected in a pre-weighed round bottom flask and then concentrated by rotary evaporation followed by pumping at full pump several times to remove remaining traces of solvent. The amount of additional oil obtained was 0.070g. This oil was then analyzed using thin layer chromatography. The sample appeared to be consistent with previous extractions of the catnip oil by conventional methods. Purification of sample was then done by column chromatography.

RESULTS and DISCUSSION

In supercritical fluid extraction (SFE), the capabilities of the solvent are dictated by temperature and pressure parameters as they affect solvent properties including density, viscosity and diffusivity. The temperature and pressure of the SF can then be modified to specifically dissolute the desired analyte. We chose to maintain a constant temperature above the critical temperature but low enough to avoid degradation of the matrix (plant material), and alter the pressure in order to maximize extraction of the catnip oil from the crushed, dry plant material. A direct proportional relationship between yield and pressure was observed. Increasing pressure increases solvent density allowing for greater permeation through the matrix. However, increasing pressure and matrix permeation decreases selectivity for the oil and results in greater quantities of impurities. According to the literature (McElvain *et al.*, 1944), oil of catnip composes .3% to .6% of the gross plant weight, while our results show yields ranging from 4.6% to 5.7% and thus contain a large amount of extraneous material. Traditional solvent extraction using diethyl ether generated material that composed 11.2% of the extracted catnip according to weight. Extractions using traditional organic solvents, due to a higher degree of polarity, draw out more impurities and result in larger, yet impure products. While SFE at the pressures studied includes a significant amount of impurities within the oil, there is a marked decrease in the amount of impurities found within the SF extracted product as compared to the oil obtained using traditional solvent extraction.

Nepetalactone, the biologically active component found in the oil of catnip, exists as two stereoisomers (Figure 1) within the *Nepeta cataria* species. NMR analysis performed on oil samples extracted using SF's indicates the presence of nepetalactone (Figure 3). Modification of extraction parameters previously mentioned can optimize the extraction of this compound.

Our study was restricted to pressures of 5500, 6000 and 9000psi. It is plausible that ideal pressure conditions exist below those examined. Additionally, temperature, a variable effecting the diffusivity of the solvent, was left absent from our consideration. The examination of extractions performed under varying temperature conditions with a constant pressure is a possibility for further experimentation.

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