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Cannabis Smoke Condensate I: The Effect of Different Preparation Methods on Tetrahydrocannabinol Levels

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***Cannabis sativa* contains more than 400 known compounds, of which the terpene chemicals, called cannabinoids, are unique to this species. The cannabinoids, which occur as the corresponding acids in the plant material, are the major psychoactive components in this species. The compounds are decarboxylated from the inactive acidic form into the active form by means of smoking. Previous research has made use of the tobacco industry's standard method and adaptations thereof to produce a cannabis smoke condensate. In this study the method of smoke production, which includes the puff frequency, puff length, and puff volume, was tested and the concentration of the major cannabinoid, Δ^9-tetrahydrocannabinol (THC), and the amount of by-products produced under the different conditions were quantified. This study aimed at combining the existing methodology and at providing quantitative results on the influence of the preparation method on the concentration of THC in the smoke. The results indicate that the method of smoke production influences the amount of THC produced (e.g., longer puff length yielding a higher amount of THC). The THC concentration in the smoke condensate varied between 22.17 mg/g of cannabis and 54.00 mg/g, while the amount of by-products produced varied between 25.57 mg/g and 107.40 mg/g.**

Cannabis sativa L. (Cannabaceae) has been used for centuries as an medicinal plant, but today it is better known as a recreational drug. Recently, renewed interest in its medicinal properties has resulted in some countries registering cannabis-derived preparations as a drug for the treatment of mainly nausea and vomiting associated with chemotherapy. Over the last several years, cannabis-based medicines such as Sativex have been investigated for the treatment of spasticity, chronic pain, disruption of sleep, and urinary dysfunction associated with multiple sclerosis and other neurological disorders (Smith, 2007). The activity of this plant is caused by cannabinoids. As far as is known, the cannabinoid THC is the most active component. There are various ways of using the plant material as a recreational drug or medicine, of which the preparation of a tea, as a baked product, and smoking are the most important. The preferred method of ingestion is by smoking the plant material. In all of these processes, heating the material plays an important role, as this will decarboxylate the naturally occuring inactive tetrahydrocannabinolic acid (THCA) into the active tetrahydrocannabinol (THC). Cannabis smoke has therefore been extensively studied in the past and various methods have been used to prepare the smoke condensate. The standardized method used by the tobacco industry to produce a tobacco smoking condensate uses a total puff volume of 35 ml, a puff duration of 2 s (volume of 17.5 ml/s), and a puff frequency of 60 s. To obtain the tobacco smoke condensate an commercially available Borgwaldt apparatus is used and the smoke is produced under ISO 4387:1991 and 2000 standards. This method has been used and adapted in order to produce a cannabis smoke condensate (Fetiman et al., 1973; Adams & Jones, 1973; Maskarinec et al., 1976; Lee et al., 1976; Van den Bosch & Salemink, 1977; Busch et al., 1978; Novotny et al., 1982; Johnson et al., 1984; Hiller et al., 1984; Lee et al., 2005).

The somewhat different preparation methods used by the researchers to produce a cannabis smoke condensate might lead to obtaining different results. In certain cases the exact method of smoke production is not adequately described (e.g., puff volume of 40 ml/s or 40 ml for the total puff length) or not all the fundamental information about the smoke condensate, like the total yield obtained, amount of cannabis used, concentration of THC, moisture content, etc., is included.

The main problem experienced with using the tobacco industry's method is that the cannabis material does not burn very well in comparison to tobacco. Researchers have therefore adapted

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the tobacco standard method and in order to overcome this problem increased the volume of suction (Adams & Jones, 1973), the frequency of suction, and the the length of suction. No literature could be found in which the different preparation method for a cannabis smoke condensate has been tested. The aim of this study was therefore to test the preparation method by using various settings on a small-scale smoking machine. The effects of the various settings on the total yield, THC content in smoke, and the amount of by-products were determined. This will give insight into the importance of the various settings to produce a smoke condensate and it might be the first step in proposing a future standardized method for the production of a cannabis smoke condensate.

MATERIALS AND METHODS

Materials

Plant Material and Chemicals

The cannabis plant material was obtained from the Office of Medicinal Cannabis and grown by Bedrocan BV (Veendam, The Netherlands) and was of the Bedrocan variety. Only the female flower tops were used. This cultivar had at the time of use a THCA content of 174 mg/g (17.4%) in dry weight plant material. All chemicals used were of analytical reagent (AR) purity, and the high-performance liquid chromatography (HPLC) solvents were of HPLC grade. THC, THCA, cannabigerol, and cannabinol standards were purchased from Farmalyse (Zaandam, The Netherlands).

Preparation of Cannabis Cigarettes

Commercial available cannabis cigarette paper (109.0 mm length, 6.0 mm radius at filter, and 12.5 mm radius at the tip) was used (Mountain High, Rotterdam, The Netherlands). The cigarettes were prepared so as to contain 700 mg of material each. The exact weight was determined for each cigarette.

Small-Scale Smoke Machine

The small-scale smoke machine consisted of two gas traps (Lenz, DIN, NS 29/32, 100 ml) with a sinterglass filter (porosity 1) to exclude any unburned solid materials from the solvent (VWR International B.V., Amsterdam). The traps were connected in series and to a smoke regulator (homemade) to control the suction length and frequency of suction. Previous results has indicated that 93% of the total yield is trapped in the first trap and the remaining 7% in the second trap (unpublished). The controller was connected to a vacuum pump. Between the controller and the trapping system an additional regulator was placed in order to control the suction volume. Before each smoking experiment the settings were tested with a stopwatch and volume meter to obtain the correct settings. The traps were filled with a 1:1 mixture of ethanol and hexane (80 ml in each trap) in order to trap the resulting smoke. The experiments were conducted at room temperature.

HPLC Analysis

An Agilent 1200 HPLC with PDA detection was used to analyze the smoke condensate samples. The HPLC method of Hazekamp et al. (2004) was used to quantify the amount of THC present in the smoke condensate by using a five-point standard curve of the THC standard. In short, the system consisted of a Phenomenex RP18(2) 150 $\times$ 4.6-mm, 5-μm column. The mobile phase consisted of 0.1% formic acid (A) and methanol/0.1% formic acid (B). The gradient system employed was: 0 min 65% B, 28 min 100% B, 30 min 100% B, 31 min 65% B, 33 min 65% B.

Methods

Moisture Content of Material and Recovery of THC

The moisture content of the material was determined by drying the material at 80°C for 1 wk. The moisture content of the material was determined to be 4.13 $\pm$ 0.06%. For the recovery experiments known amounts of THC (at concentrations similar to which were trapped during the smoking experiments) were added into the trap system and recovered as with the smoke samples. The recovery was found to be 99.5 $\pm$ 5.2%

Variation in Preparation Method

Three variables were tested during these experiments. The samples were prepared by subdividing the samples into three groups corresponding to the variables. In group A the puff frequency of 60, 30, and 15 s was tested. After each group was tested the settings that resulted in the best burning efficiency, and that in fact closely corresponded to the tobacco industries standards, were used during the analysis of the next group. Group B contained the samples that were prepared with a varying puff length of 2, 3, and 4 s. Group C contained the variation of the total puff volume of 25, 35, 45, and 50 ml. For each setting five cigarettes were smoked.

The following analysis were performed in Group A:

Setting 1: 35 ml (total puff volume) for 2 s (puff length) every 60 s. (puff frequency). This is the tobacco industries standard method.
Setting 2: 35 ml for 2 s every 30 s.
Setting 3: 35 ml for 2 s every 15 s.

Group B:

Setting 1: 35 ml for 2 s every 30 s.
Setting 2: 35 ml for 3 s every 30 s.
Setting 3: 35 ml for 4 s every 30 s.

Group C:

Setting 1: 25 ml for 3 s every 30 s.
Setting 2: 35 ml for 3 s every 30 s.
Setting 3: 45 ml for 3 s every 30 s.
Setting 4: 50 ml for 3 s every 30 s.

Cannabis Smoke Production

The cannabis cigarettes were fitted into the glas filter system and were sealed with parafilm. After the cigarettes were lit by hand the smoke was trapped by the double solvent trap at room temperature. The distance for the smoke to travel between the cigarette and the solvent was 25 cm. The cigarettes were smoked up until the butt, after which the butt was removed and the glass filter was washed with ethanol. This was done in order to dissolve any components attached on the glass surface. The trapped smoke was transfered to a round-bottom flask and dried on a rotary evaporator at 40°C. The samples were stored at −20°C until HPLC analysis was performed.

RESULTS AND DISCUSSION

THC Concentration

Figure 1 gives the concentration of THC in the smoke condensate, the amount of by-products formed, and the total yield as produced under the different settings. The by-products also contained trace amounts of two other cannabinoids, cannabigerol and cannabinols which were identified with co-injection with authentic standards. During the production of the smoke condensate only the physical parameters of smoke production were investigated. The types of solvent traps that were used were not investigated during these experiments. The choice of a mixture of ethanol and hexane was decided based upon previous work

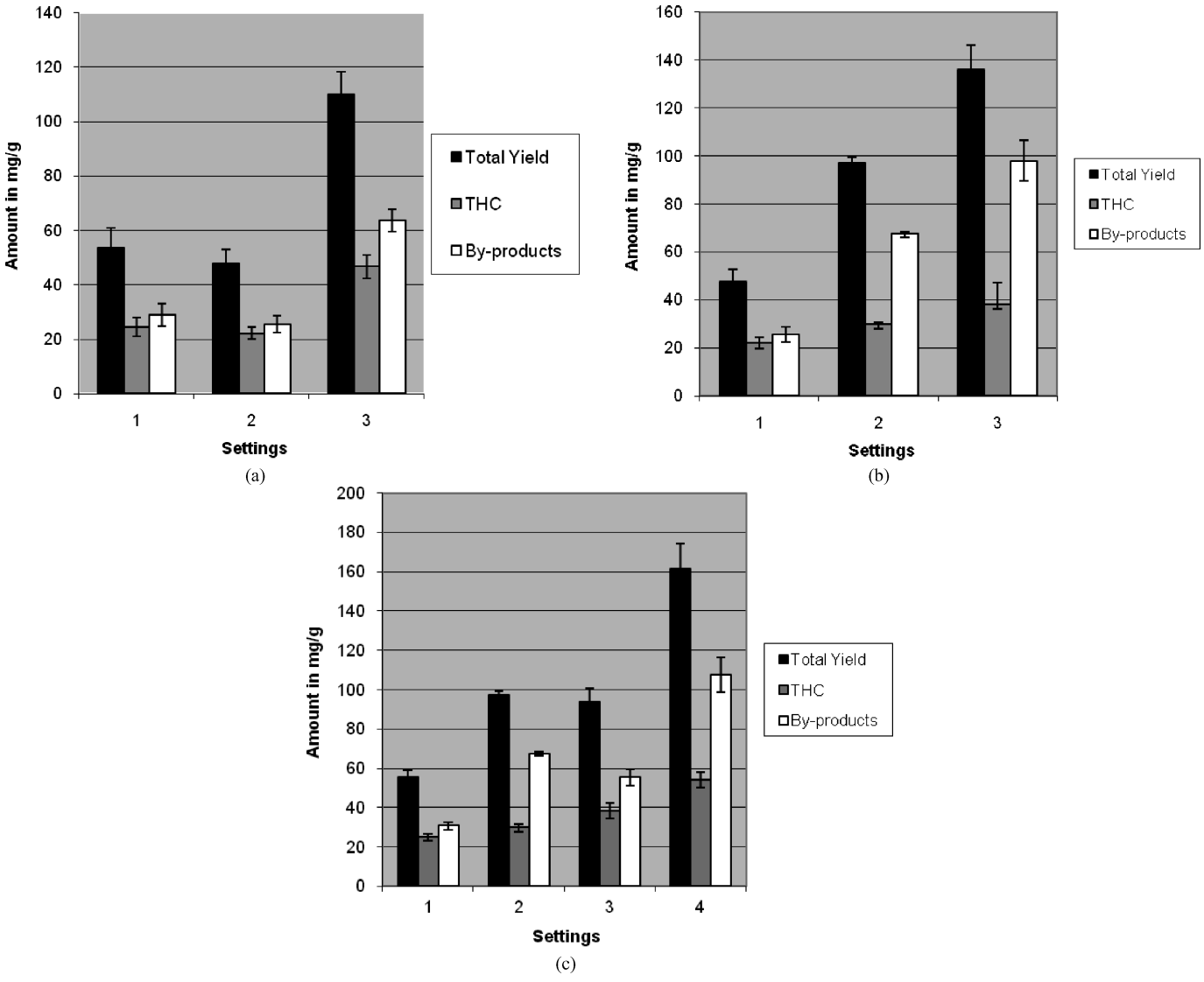

FIG. 1. (a) Smoke condensate produced by altering the frequency of suction (group A) between 60 s (setting 1), 30 s (setting 2) ,and 15 s (setting 3). (b) Smoke condensate produced by altering the length of suction (group B) between 2 s (setting 1), 3 s (setting 2), and 4 s (setting 3). (c) Smoke condensate produced by altering the volume of suction (group C) between 25 ml (setting 1), 35 ml (setting 2), 45 ml (setting 3), and 50 ml (setting 4).

carried out in our laboratories. Different solvents and methods of trapping (filter and solvent traps) were investigated (unpublished). The hexane–ethanol mixture resulted in the highest overall yields obtained, while the recovery of THC with the use of this system was also found to be adequate.

Variation in the puff frequency between 60, 30, and 15 s yielded a variation in THC yield of –11.9% and 47.4% compared to the tobacco industry's 60-s puff frequency. It was expected that the THC concentration would increase with the reduction in time of the puff frequency. The similar result obtained for the puff frequencies of 60 and 30 s was therefore not expected. The percentages of THC compared to the total yield at the different settings were calculated to be 45.8%, 46.4%, and 39.6%, respectively, at the 3 different settings. This indicates that the puff frequencies of 60 and 30 s yielded very similar results while the amount of by-products produced at a puff frequency of 15 s increased slightly. This trend can be seen when the total yields are compared as well. The total yields obtained from settings 60 and 30 s almost doubled at the setting 15 s. A possible explanation for this is that the average temperature of the cigarette remains higher when a puff every 15 s is used.

The results obtained from varying the puff length indicated that the THC content increased with the length of the puff. The increase of THC concentration for the puff lengths of 3 and 4 s was 25.5% and 41.8%, respectively, compared to the 2-s puff. The percentage of THC compared to the total yield was calculated to be 46.4, 30.6, and 28.0%, respectively, for 2, 3, and 4 s. This indicates that the puff length will cause the concentration of THC in the smoke condensate to decrease from about half (46.4%) at a 2-s puff to about a quarter (27.9%) at a 4-s puff. It can also be concluded that the puff length has a large effect on the formation of by-products.

The THC concentration in the smoke condensate increased as expected with an increase of puff volume. The increase compared to a total puff volume of 25 ml was 16.3, 36.5, and 54.1%, respectively, for the 35-ml, 45-ml, and 50-ml puff volumes. The total THC concentration compared to the total yield was found to be 44.7, 30.6, 40.8, and 33.6%, respectively.

The results indicate that the THC content in the smoke condensate will increase if the puff frequency is shortened and the puff length and volume are increased. The exception to this trend seems to be the reduction of the puff frequency from every 60 s to every 30 s. The THC content remained similar at both these settings. To be able to establish an easy-to-use and reproducible smoking method the standard deviation and the burning efficiency should be considered. The preperation of the smoke condensate under the tobacco industries standards took more than 45 min per cigarette. At 30 s and 15 s the cannabis burns relatively well. The puff length also played a role in the burning efficiency. The longer the puff length, the better the material burned. A puff length of 3 s was seen as sufficient to produce a constant burning of the cigarette.

The puff volume also had the expected effect on the increase of the THC in the smoke condensate. The higher the volume, the better the cigarette burns, which will also lead to higher THC levels. From these analysis we found that the burning efficiency was adequate at the following settings: a puff frequency of 30 s, puff length of 3 s, and a total puff volume of 35 ml.

CONCLUSIONS

During our analysis we have found that the settings at which the cannabis condensate are produced are quite reproducible based on the standard deviations between the five replicates tested at each setting. We have also found that a slight change in the method of preparation has a large influence on the amount of THC in the smoke condensate, while the amount of by-products produced at the different settings had an unexpected result. This will indicate that the way in which users smoke cannabis will have a large influence on the amount of THC and by-products inhaled.

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