

***Cannabis sativa* L.: Effect of drying time and temperature on cannabinoid profile of stored leaf tissue**

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Abstract

Leaf tissue of *Cannabis sativa* L. was subjected to several time-temperature régimes before extraction and analysis for cannabicyclol, cannabidiol, Δ^9 tetrahydrocannabinol, and cannabinol. Differences in time-temperature treatments resulted in significant variations in cannabinoid content of leaf tissue. The need for uniformity in tissue-preparation methods is discussed.

Cannabis sativa L. has been used as an intoxicant in the United States for more than seven decades (1). Increased cannabis consumption has resulted in intensified scientific investigations of the narcotic characteristics of the species. Cannabinoids are now being extracted and analysed by laboratories affiliated with universities, forensic chemists, and Federal research agencies.

Cannabinoid analysis involves extraction of plant tissue with an organic solvent, concentration of extract, and subsequent analysis by chromatographic methods. There is little uniformity in methods of plant-tissue preparation before cannabinoid extraction. For example, Carew (2) dried cannabis leaf tissue at 40 °C. Lerner (3) and Turner and Hadley (4) used air-dried tissue. Phillips *et al.* (5) dried tissue for 30 minutes at 85-90 °C.

Variation in moisture content of plant tissue is caused by age, leaf location, environment, and other factors. To compare cannabinoid content of plant tissues by weight, moisture content must be made as uniform as possible without destruction of the cannabinoid components. This paper reports the effects of oven-drying *C. sativa* leaf tissue at several time-temperature periods. Weight loss and variations in cannabinoid content of the heated and unheated leaf tissue samples were compared. This report describes a uniform method of plant-tissue preparation for cannabinoid analysis.

Eight-week-old greenhouse-grown (6) plants of *C. sativa* L. of Afghan seed (7) were stripped of their leaves. The leaf material was air-dried for 24-48 hours, then ground to pass a 60-mesh sieve, uniformly mixed, and stored in darkness at ambient temperature.

Three subsamples of ground leaf tissue were used for each of the following treatments: 1, 4, 16, and 64 hours at 65 °C, 85 °C, and 105 °C. After exposure to the above time-temperature régimes, the samples were placed in a desiccator containing activated silica gel to equilibrate with ambient temperature. Weighings before and after oven treatments allowed comparisons of the relative weight losses. Check treatments were subjected to the same procedure as the heated treatments, exclusive of heat.

Cannabinoids were extracted and analysed by a modification of the procedure of Turner and Hadley (4). Modifications involved chromatograph temperatures,

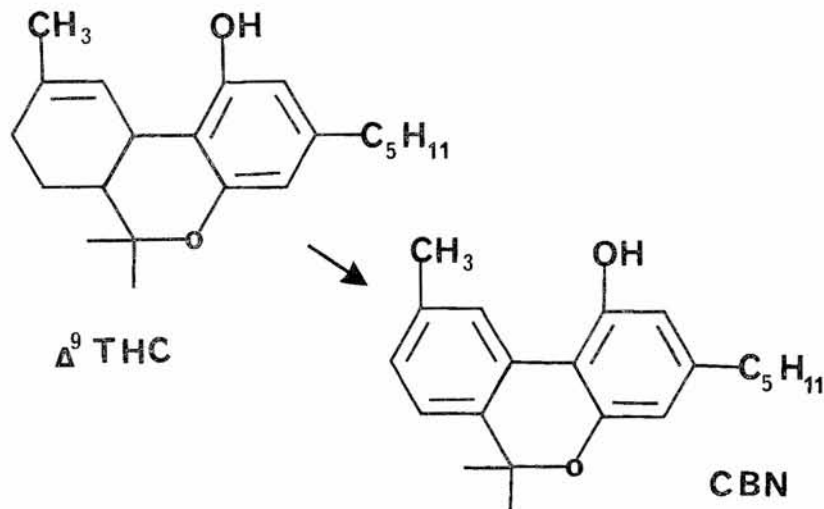
stationary phase, and method of introduction of internal standard (8). Nitrogen carrier gas was used with a Barber-Coleman (9) model 5005 gas chromatograph (10) equipped with a H-flame ionization detector (10).

The effects of the various time-temperature treatments on the content of cannabicyclol (CCC), cannabidiol (CBD), Δ^9 -tetrahydrocannabinol (Δ^9), and cannabinol (CBN) are presented in table 1.

There were no differences in the CCC content of the tissue within the 65 °C treatment (table 1). Decreasing quantities of CCC were associated with the two longer exposure times at 85 °C and 105 °C. No CCC was detected after 64 hours exposure at 105 °C.

A similar pattern of decreasing cannabinoids with increased exposure time at 85 °C and 105 °C was found for CBD (table 1). This response to time and temperature is also illustrated by Δ^9 values.

CBN values increased with time at 105 °C. This probably reflected the destruction of Δ^9 .



Farnsworth indicated (11) that cannabinoid conversion can occur with time in stored material. He stated that conversion was more rapid in tropical areas than in temperate areas, implying heat enhancement of the conversion process.

To increase our confidence in the results of the 16-hours, 65 °C portion of the time-temperature study, a mixture of CCC, CBD, Δ^9 , and CBN was prepared (12). A part of this mixture was subjected to 65 °C for 16 hours, while the other portion was refrigerated. Gas chromatograph analysis indicated no cannabinoid degradation by this treatment.

Table 2 shows the average weight loss of cannabis leaf tissue resulting from time-temperature treatments. The general trend was for an increased weight loss as temperature and/or time of exposure increased. No significant differences in weight loss were noted for the 1-hour exposures at 65 °C and 85 °C, or for 4 and 16 hours at 85 °C. Weight loss decreased for the tissue exposed at 65 °C for 64 hours relative to the weight losses for the other time exposures at the same temperature. This portion of the experiment was repeated (in time) with similar results. The precise explanation of this phenomenon has not yet been ascertained. Several hypotheses are proposed: First, exposure at 65 °C for 64 hours may have created a hygroscopic component in the tissue,

TABLE 1

CCC, CBD, Δ^9 , and CBN content in ppm of cannabis leaf tissue subjected to several time-temperature treatments*

Cannabinoid	Temperature		Times (hours)			
	°C		1	4	16	64
CCC	65		58.3ab	70.0a	60.7ab	61.7ab
	85		51.7ab	55.0ab	48.3b	31.7c
	105		57.3ab	56.7ab	30.0c	0d
	Control	65.0ab				
CBD	65		733ab	818a	740ab	676bc
	85		733ab	686bc	641bc	618c
	105		721ab	605c	688bc	480d
	Control	810a				
Δ^9	65		763ab	775ab	695abc	616cd
	85		720ab	690bc	580d	378e
	105		680bc	610cd	445e	147f
	Control	790a				
CBN	65		345abc	468abc	431abc	395abc
	85		483abc	341abc	531ab	441abc
	105		361abc	321bc	580ab	616a
	Control	216c				

* Values not followed by the same letter (within cannabinoid) are significantly different at the 1 per cent level (13).

TABLE 2

Percentage mean weight loss of cannabis leaf tissue as a result of exposure to time-temperature treatments^a

Temperature °C	Times (hours)			
	1	4	16	64
65	7.58g ^b	7.92f	8.16e	7.08h
85	7.39g	9.58cd	9.77c	10.28b
105	9.43d	9.45d	10.46b	11.00a

^a Percentage loss compared to weight of air-dried tissue.

^b Values not followed by same letter are different at the 1 per cent level (13).

which had a greater affinity for moisture than the desiccant, thus sorbing moisture while equilibrating at ambient temperature. Further heating at higher temperatures apparently destroyed the hygroscopic characteristics of the unknown moiety. Secondly, an oxidation reaction may have been induced by the particular treatment, resulting in a weight gain. Studies have begun to determine an explanation for this occurrence.

In summary, various time-temperature treatments have resulted in significant differences between cannabinoid constituents of cannabis leaf tissue. The differences are attributed to: (a) differences in dry weights resulting from different treatments, and (b) time-temperature effects on the integrity of specific cannabinoids. These results indicate that valid comparisons of cannabinoid profiles cannot be made without establishing uniformity in tissue preparation. Uniformity could be established by: (a) appropriate drying methods, or (b) use of uniform relative-humidity environments for maintenance of tissue at known moisture levels. Sixteen hours at 65 °C was the most appropriate drying treatment used in this investigation.

References and notes

1. "National Commission on Marihuana and Drug Abuse". 1972. *Marihuana: A signal of misunderstanding*. U.S. Government Printing Office, Washington, D.C.
2. Carew, D. P. 1971. *J. Forensic Sciences*, 16:1, 87-91.
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4. Turner, C. E., and K. Hadley. 1973. *J. Pharm. Sci.* 62:2.
5. Phillips, R., R. Turk, A. Mammo, N. Jain, D. Crim, and R. Forney. 1970. *J. Forensic Sci.* 15:2, 191-200.
6. "Plants were grown in Maryland during December and January 1972 and 1973". BNDD Analytical Lab. No. PU0084880, Schedule I No. PU0059659.
7. P. I. Number 378939.
8. Inlet temperature was 280°C, column 240°C, and detector 280°C. Internal standard was 4-androstene-3, 17-dione, contained in ethanol solution used to dissolve the concentrated tissue extract before its injection into GC. Three-per cent OV-17 was used instead of 2 per cent OV-17. The support was chromosorb WHP, 100-120 mesh.
9. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.
10. Turner and Hadley used a mass spectrometer which was not used for this study.
11. Farnsworth, N. R. 1969. *J. Am. Pharm. Assn.* 410-414.
12. Standards obtained from NIMH.
13. Duncan's new multiple-range test.