Influence of Storage Conditions on the Chemical Potency of Herbal Cannabis

IRENNE GABRIELA TROFIN[1]*, CORINA CLAUDIA VLAD[1], GABRIEL DABIJA[1], LAURENŢIU FILIPESCU[2]

[1] General Inspectorate of Romanian Police, Central Laboratory for Drug Analysis and Profiling, 6 Mihai Voda, 050041, Bucharest, Romania

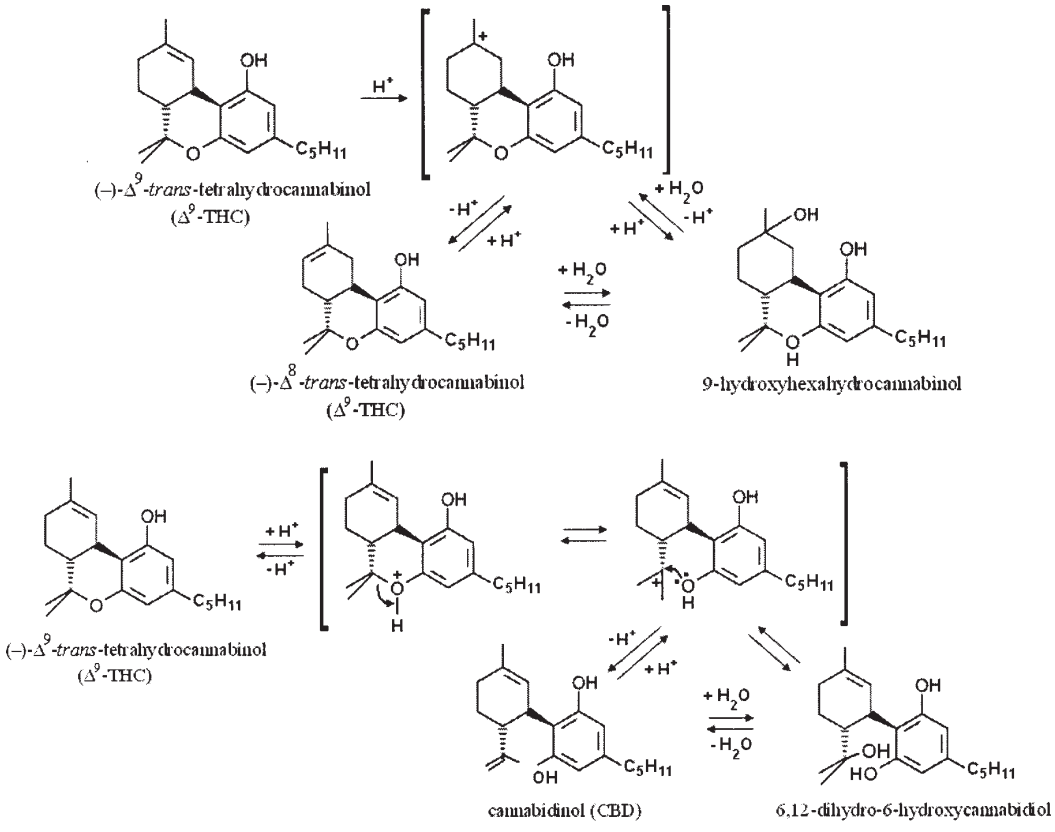
[2] University "Politehnica" of Bucharest, Faculty of Applied Chemistry and Materials Science, Department Technology of Inorganic Substances and Environmental Protection, 313 Splaiul Independentei, 060042, Bucharest, Romania

The aim of the present paper was to investigate the stability of cannabinoids in herbal cannabis upon long-term storage. The content of tetrahydrocannabinol (Δ^9-THC), cannabinol (CBN), and cannabidinol (CBD) in herbal cannabis from ten different regions of the world were measured for up to four years of storage in darkness at 4°C and in natural light of laboratory at 22°C. The degradation of Δ^9-THC was faster in the first year than in subsequent years, and more pronounced for the samples exposed to light at 22°C than those stored in darkness at 4°C. The content of CBN increases during the storage and the increase is more pronounced for the samples exposed to light at 22°C than those stored in the darkness at 4°C. These results are consistent with those obtained for Δ^9-THC. Also, a new criterion for the chemical potency ranking in different herbal cannabis grades was approached on the basis of the Δ^9-THC degradation kinetics.

Keywords: cannabis, chemo-type, potency, cannabinoid

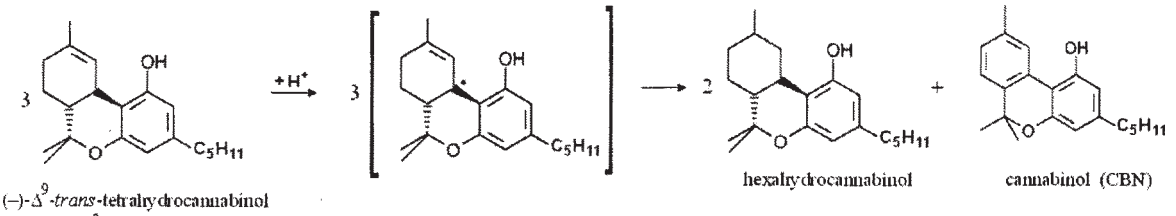
Herbal cannabis (*Cannabis sativa L.*) produces a specific class of terpenophenolic compounds called cannabinoids [1-5]. The content of the major cannabinoids, (–)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN) in different Cannabis plants can be related to their regions of origin [6-8]. Also, Cannabis plants are classified by their chemical phenotype or chemo-type as drug-type or fiber-type plants, taking into account either the overall amount of Δ^9-THC produced, or the THC/CBD and the (THC+CBN)/CBD ratios [9-11].

The major psychoactive chemical compound in cannabis, Δ^9-THC, is relatively unstable and may undergo changes when the herbal cannabis is storage long term. Thus, as a function of environmental factors, Δ^9-THC can be converted to isomeric (–)-Δ^8-*trans*-THC by migration of the double bound and to cannabidinol by hydrolysis of the ether linkage [12-15].



* email: irennetrofin@yahoo.com; Tel.: 0722495004

Furthermore, degradation of Δ^9-THC is very fast in acidic solutions [15]. In the presence of air Δ^9-THC is oxidized to cannabinol [16]. Also, its instability to heat and light has been reported [17-19].



(–)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC)

hexahydrocannabinol

cannabinol (CBN)

The objective of this paper is to investigate, based on experimental results regarding the content of the major cannabinoids, the influence of storage conditions such as temperature and light on the chemical potency of cannabis plants. Chemical potency is quantified by the content in the major psychoactive component of the herbal cannabis, namely Δ^9-THC. This paper also focuses on endorsing the specificity of the chemical phenotype of Cannabis plants from different geographic areas.

Experimental part

Chemicals and reagents

All chemicals and reagents used for samples preparation and analysis were of analytical grade from Merck (Darmstadt, Germany). The standard grade samples of Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN) were purchased from Lipomed, Arlesheim, Switzeland. The ultrapure water used in HPLC analyses was prepared in-house using a Millipore system, model Milli-Q Integra 3.

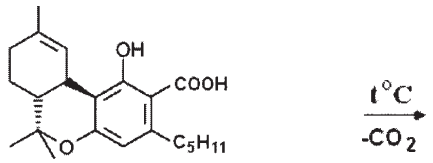
Cannabis samples

Cannabis sativa L plants from 10 different geographic regions of the world (for security reasons the regions have been marked with symbols: C1-C10) seized by criminal prosecution authorities from Romania and provided by Central Laboratory for Drug Analysis and Profiling were subject to experimental investigation. Fresh samples of 110 cannabis plants (11 plants for each region) were dried for 96 h in a 25°C forced ventilation oven. The dried material was then coarsely selected to separate the flowers (used in further experiments) from the rest of the plant. Crumbly floral samples were then manually grinded, sieved (mesh size 710 μm), initially analyzed and stored under different conditions. Thus, an equal number of samples were stored both in the darkness at 4°C and in the natural light of laboratory at 22°C for four years. At regular intervals (every three months), samples were taken for the analysis of major cannabinoids (Δ^9-THC, CBD, and CBN).

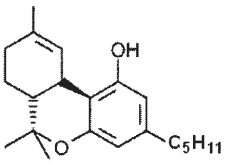
Methods

The sample preparation for analyses was performed according to the below procedure. In brief, 0.2 g of grinded sample was extracted with 20 mL of a methanol-chloroform (9:1, v/v) mixture. To extract the analytes the sample was shaken for 30 min and then placed in an ultrasonic bath at ambient temperature for 15 min. The extract was filtered and an aliquot sample (0.6 mL) of filtrate was transferred to a 4 mL vial and then evaporated to dryness by oven evaporation at 80°C, in order to prevent any decomposition reactions. After this, the vial was put into a heating unit at 220°C for 12 min , where the traces of tetrahydrocannabinolic acid (THCA) are decarboxylated. Decarboxylation is required to determine the entire content of Δ^9-THC of the sample.

Before analyses, the residue was extracted in 1.5 mL of the extraction solvent (methanol-chloroform 9:1, v/v). After this, the sample was subjected to analyses of the major cannabinoids (Δ^9-THC, CBD, and CBN) content [20].



(–)-Δ^9-*trans*-tetrahydrocannabinol acid (Δ^9-THCA)



(–)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC)

Analytical protocol

Extracts obtained by procedure described above have been subject to analytical investigations through instrumental methods (GS-MS – Gas Chromatography-Mass Spectrometry and HPLC – High-Performance Liquid Chromatography) in order to find out the content in major cannabinoids such as Δ^9-THC, CBD, and CBN.

GC-MS analyses were carried out on a 6890N gas chromatograph coupled to a 5973N mass selective detector (Agilent, Waldbronn, Germany) and a CTC Combi-PAL autosempler (Chromtech, Idstein, Germany). Separation was achieved on a fused silica capillary column (HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness). Temperature program: 150°C hold for 2 min, 10°C/min to 280°C, hold for 5 min. The injection port and interface temperature were 290 and 300°C, respectively. Splitless injection mode was used, and also, helium was used as carrier gas with a flow rate of 1.0 mL per minute. The scan range of MS (*m/z*) was in the range 40-450 atomic mass units (AMU) under electron impact (EI) ionization detector (70 eV).

HPLC analyses were carried out on an Agilent 1100 Series HPLC chromatograph (Agilent, Waldbronn, Germany) equipped with a quaternary pump, auto-sampler, column oven and diode-array detector (DAD) UV Lamp ON (223 nm). Chromatography was achieved on a 250 mm × 4.6 mm i.d., 5 μm Hypersil ODS column. The HPLC operates with constant flow at 1 mL mobile phase (acetonitrile 37.5% and ultrapure water) per minute.

Results and discussions

Chemical phenotype assessment

Analyses performed indicate that the cannabinoids content in cannabis plants from different regions of the world is widely varying. The variation of cannabinoid contents in the samples may be a result of environmental

Table 1
THE INITIAL MEAN CONTENT OF MAJOR CANNABINOIDS

Cannabinoid, %	Region*									
	C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
Δ^9-THC	19.88	17.67	10.61	8.11	7.89	7.44	7.31	1.55	1.18	0.29
CBN	0.94	0.88	0.63	0.08	0.12	0.25	0.22	0.04	0.37	0.01
CBD	0.52	0.12	0.74	1.58	1.33	0.96	2.37	2.18	3.78	0.24

*were marked from C1 to C10 in order of decreasing concentration in Δ^9-THC

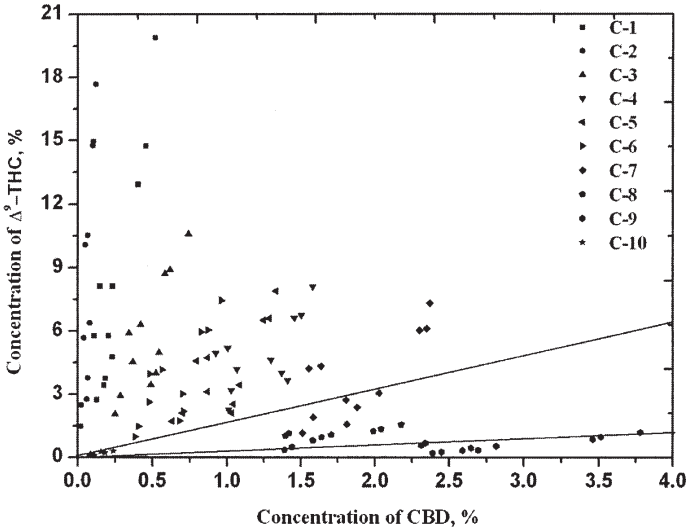


Fig. 1 Plot Δ^9-THC *vs.* CBD content of the 110 cannabis plants from 10 different regions

conditions such as climate, sunlight intensity and sunny day per year, the quality of soil in the cultivation field, altitude, etc. As a function of the amount of psychoactive components, the cannabis plants may be classified as fiber-type or drug-type.

The initial samples mean content in major cannabinoids, corresponding to each region is presented in table 1 (according with the above analyses). The percentage of Δ^9-THC content in the analyzed cannabis plants varies between 0.29% and 19.88%. As it shown in table 1, there is only one type of cannabis plants (from region C10) that has a content of Δ^9-THC lower than 0.3%. According to the UE guideline [20, 21] it can be classified as fiber-type. Thus, the results suggest that 90% of the cannabis plants from nine regions (C1-C9) can be classified as drug-type and 10% coming from one region (C10) can be classified as fiber-type. The percentage content in CBN and CBD lays in 0.01%-0.94% and 0.12% -3.78% ranges, respectively.

Based on the Δ^9-THC/CBD ratio, three chemical phenotypes or chemotypes of cannabis plants can be identified. Chemotype I or drug-type plants have a high THC/CBD ratio (much higher than 1), chemotype II or intermediate-type plants have an intermediate ratio (close to 1), and chemotype III or fiber-type plants have a low THC/CBD ratio (much lesser than 1), [8]. A plot of THC content against CBD content reveals these chemotypes. As it shown in figure 1, the cannabis plants from region C9 can be classified as fiber-type (mean THC/CBD ratio of 0.31) and the cannabis plants from region C10 (mean THC/CBD ratio of 1.24) together with those from C8 (mean THC/CBD ratio of 0.71) can be classified as intermediate type. Although the half of cannabis plants from region C7 appears in figure 1 as intermediate-type, the mean of THC/CBD ratio is much higher than 1.0 (mean THC/CBD ratio of 3.08) and these plants can be classified as drug-type. All of others cannabis plants from the rest of regions can be classified as drug-type.

The ratio (THC+CBN)/CBD or phenotypic index has been also used for distinguishing between the different phenotypes of cannabis plants. As it shown in table 2, the phenotypic index of cannabis plants from eight regions (C1-C7, C10) is higher than 1.0, while only the cannabis plants from two from regions (C8, C9) have the phenotypic index less than 1.0. These results suggest that 80% of analyzed cannabis plants are drug-type and 20% are fiber-type.

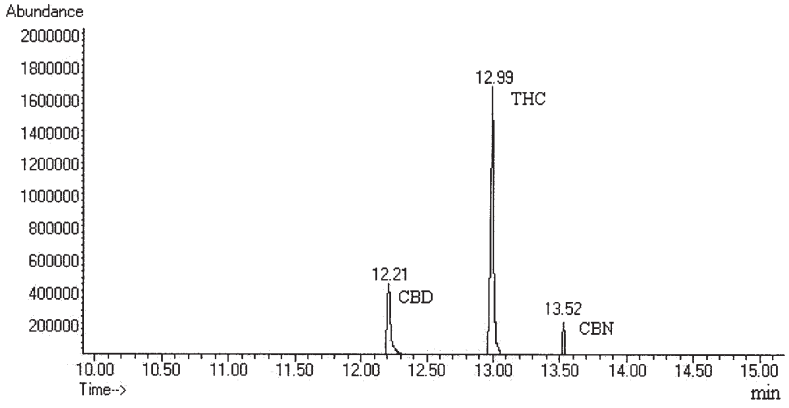
There is a conflicting classification of cannabis chemotype in plants from C8, C9, and C10 as a function of the phenotypic indicator used. Thus, when only the Δ^9-THC content is used to distinguish between the phenotypes of cannabis plants, the samples from region C10 appear as fiber-type (Δ^9-THC content less than 0.3%). When the THC/CBD ratio and phenotypic index are used as phenotypic indicators, samples from region C8 and C9 appear as fiber-type (both indicators have values less than 1.0). Independently of this contradiction, the Δ^9-THC content indicates that the samples from region C10 are fiber-type (values less than 0.3%) and the samples from regions C8 and C9 are intermediate-type (values much higher than 0.3%). Based on these results, it can be concluded that 70% of all samples (C1-C7) can be classified as drug-type, 20% as intermediate-type (C8 and C9) and 10% as fiber-type (C10).

Chemical potency of herbal cannabis as a function of storage conditions

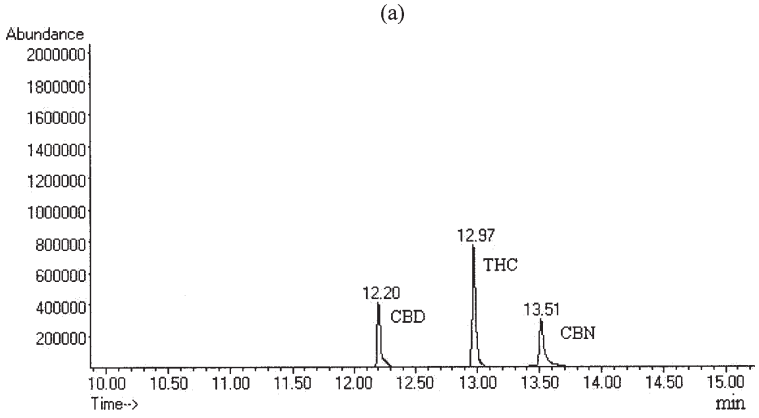
There are large differences in the variation of mean herbal cannabis potencies under certain particularly

Cannabinoid, %	Region									
	C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
Δ^9-THC	19.88	17.67	10.61	8.11	7.89	7.44	7.31	1.55	1.18	0.29
Phenotypic Index	40.4	154.58	15.19	5.18	6.02	8.01	3.18	0.73	0.41	1.28
Chemotype	I	I	I	I	I	I	I	II	II	III

Table 2
CHEMICAL PHENOTYPE OF CANNABIS PLANTS



(a)



(b)

Fig. 2 Gas gromatograms of drug-type cannabis samples (C1) stored in darkness at 4°C (a) and in natural light of laboratory at 22°C (b)

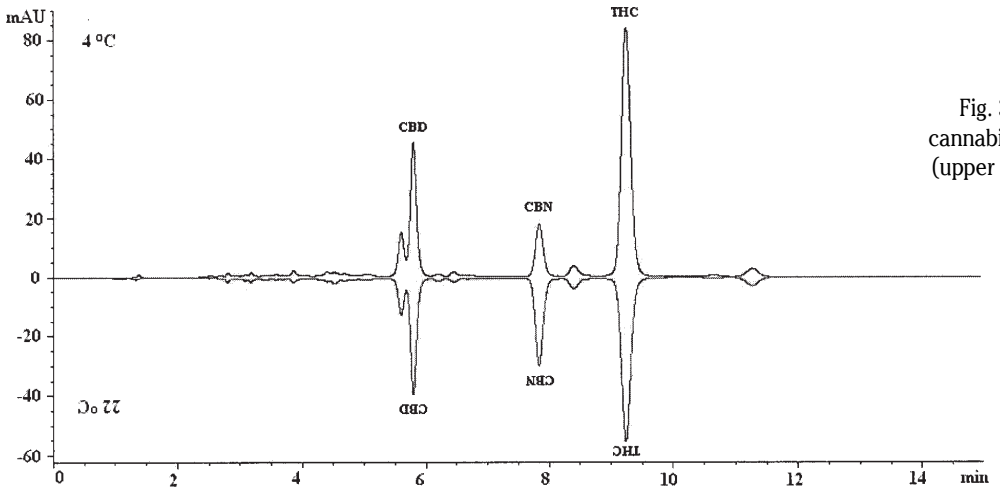


Fig. 3 HPLC chromatograms of drug-type cannabis samples stored in darkness at 4°C (upper side) and in laboratory natural light at 22°C (down side)

storage conditions. For example, after one year, the samples of herbal cannabis from region C1 (drug-type) stored in the darkness at 4°C had significantly higher Δ^9-THC mean content (10.88%) than the samples stored in the natural light of laboratory at 22°C (8.73%). Chromatograms obtained for this example are provided in the figure 2 and 3.

Figures 4-6 show the variation of major cannabinoids content in cannabis plants as a function of time and storage conditions. As it can be seen, in all cases, the Δ^9-THC content decreases during storage and it is always higher in the samples stored in the darkness at 4°C than in the samples stored in the natural light of laboratory. On the contrary, the CBN content increases during storage and is always higher in the samples stored in natural light of laboratory than in the samples stored in darkness at 4°C. The results obtained for all samples from all regions regarding the evolution of major cannabinoids content during storage under different conditions are presented in table 3. The same trend as in examples shown above was observed in all cases.

The results suggest a faster degradation of Δ^9-THC in the first year than in subsequent years. Furthermore, the degradation of Δ^9-THC in the samples exposed to light at 22°C is more pronounced than in the samples stored in the darkness at 4°C. Thus, in the case of samples from region C1 stored in the darkness at 4°C, 45.23% of Δ^9-THC was lost in the first year with a loss average of 11.31% every three months, 20.26% in the second year with a trimestrial loss average of 5.06%, 14.65% in the third year with a trimestrial loss average of 3.66%, and 5.17% in the fourth year with a trimestrial loss average of 1.29%. In the case of samples from the same region but stored in the natural light of laboratory at 22°C, 56.06% of Δ^9-THC was lost in the first year with a loss average of 14.01% every three months, 19.64% in the second year with a trimestrial loss average of 4.91%, 7.71% in the third year with a trimestrial

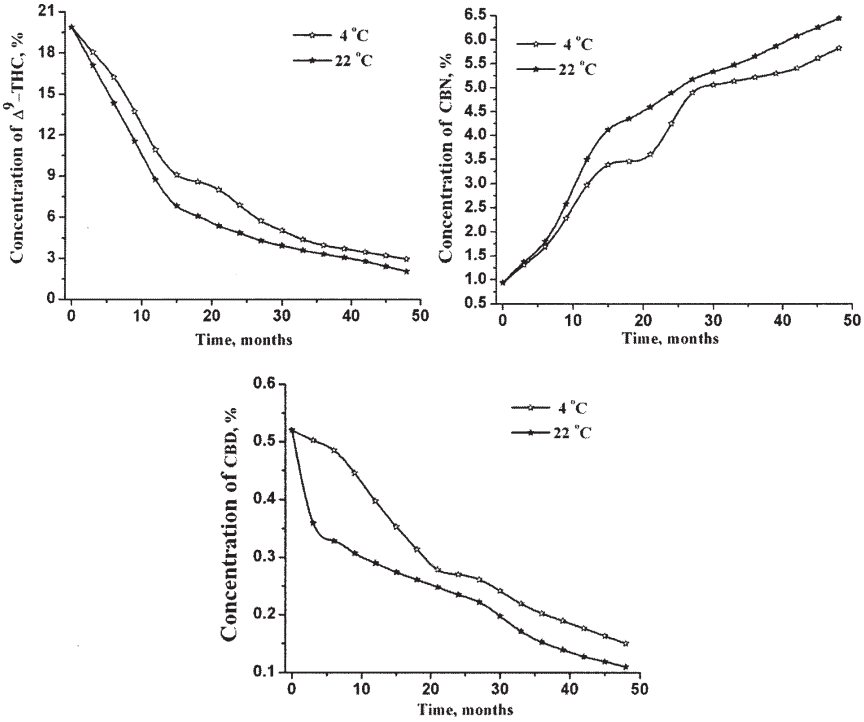


Fig. 4 Time variation of cannabinoids content in drug-type cannabis plants (region C1)

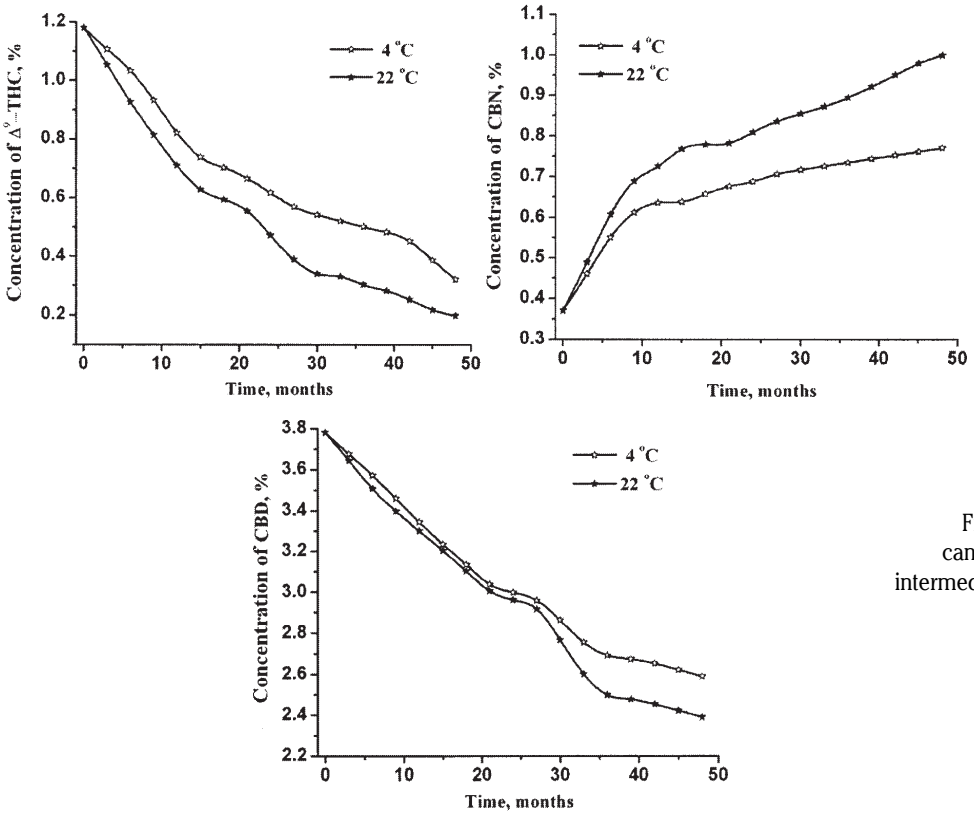


Fig. 5 Time variation of cannabinoids content in intermediate-type cannabis plants (C9)

loss average of 1.93%, and 6.37% in the fourth year with a trimestrial loss average of 1.59%. Finally, after four years, the samples stored in the darkness at 4°C lost 85.31% of Δ^9-THC and the samples stored in the natural light of laboratory at 22°C lost 89.79% of Δ^9-THC (with more 4.48%), but the patterns of these losses were significantly different. These results clearly highlight that degradation of Δ^9-THC is influenced by storage conditions such as temperature and light.

Currently, it is accepted that CBN is the primary product of Δ^9-THC degradation [16]. But, the experimental results confirmed that 100% conversion of Δ^9-THC in CBN is really not taking place. In the case of samples from region C1 stored in the darkness at 4°C, the CBN content increased with 72.49% in the first year and then with 5.39% in the second year, 4.01% in the third year, and 1.87% in the fourth year. In the case of samples exposed to light at 22°C, the CBN content increased with 73.1% in the first year, 7.65% in the second year, 2.61% in the third year, and 2.03% in the fourth year. This change in the CBN content cannot be directly correlated only with the biodegradation of the Δ^9-THC. It appeared that other degradation routes (chemical degradation) may contribute to the overall increase in CBN content upon long-term storage.

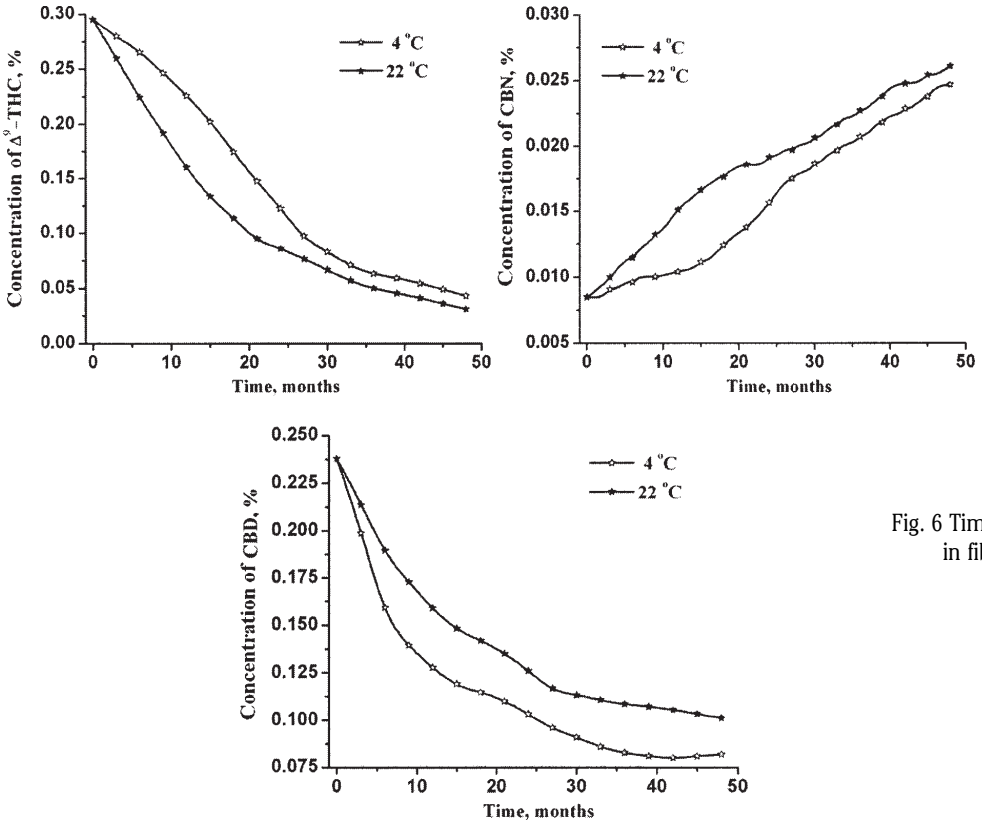


Fig. 6 Time variation of cannabinoids content in fiber-type cannabis plants (C10)

Cannabinoid %	Time years	Region									
		C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
Δ^9-THC	1	10.89	13.28	7.88	5.97	5.82	5.20	5.42	1.25	0.82	0.22
	2	6.86	8.89	5.57	5.05	4.35	3.68	3.76	1.12	0.62	0.12
	3	3.95	5.12	4.64	4.30	2.96	2.71	2.85	1.03	0.50	0.06
	4	2.92	2.77	3.45	3.68	2.11	1.73	1.57	0.81	6.32	0.04
CBN	1	3.42	3.02	1.45	0.86	1.01	1.03	1.00	0.11	0.64	0.01
	2	4.25	5.08	1.91	1.10	1.59	1.19	1.34	0.12	0.69	0.01
	3	5.21	5.85	2.26	1.59	1.94	1.59	1.67	0.14	0.73	0.02
	4	5.82	6.28	2.47	1.95	2.28	2.02	2.03	0.17	0.77	0.02
CBD	1	0.40	0.09	0.57	1.41	1.23	0.82	2.28	1.97	3.34	0.13
	2	0.27	0.09	0.56	1.28	1.12	0.69	2.12	1.82	3.00	0.10
	3	0.20	0.08	0.54	1.34	1.06	0.72	1.95	1.67	2.69	0.08
	4	0.15	0.06	0.49	1.41	1.03	0.68	1.81	1.59	2.59	0.08

Table 3
EVOLUTION OF THE MAJOR CANNABINOIDS CONTENT DURING STORAGE IN THE DARKNESS AT 4°C

Cannabinoid %	Time years	*Region*									
		C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
Δ^9-THC	1	8.73	12.99	7.59	5.71	5.67	5.07	5.30	1.11	0.71	0.16
	2	4.83	8.34	5.23	4.82	4.15	3.47	3.61	0.97	0.47	0.08
	3	3.30	4.28	3.86	3.67	2.53	2.10	2.36	0.67	0.30	0.05
	4	2.03	1.48	2.05	2.28	1.71	0.98	1.17	0.36	0.20	0.03
CBN	1	3.49	1.11	1.58	1.01	1.12	1.18	1.14	0.21	0.72	0.01
	2	4.88	5.31	2.08	1.29	1.77	1.36	1.52	0.25	0.81	0.02
	3	5.65	6.18	2.47	1.81	2.15	1.85	1.89	0.30	0.89	0.02
	4	6.44	7.11	2.94	2.45	2.63	2.47	2.28	0.42	0.10	0.03
CBD	1	0.34	0.08	0.51	1.35	1.16	0.76	2.22	1.92	3.30	0.16
	2	0.23	0.06	0.43	1.15	1.00	0.56	1.99	1.78	2.96	0.13
	3	0.15	0.03	0.33	1.04	0.76	0.42	1.66	1.48	2.50	0.11
	4	0.11	0.02	0.25	1.01	0.63	0.38	1.51	1.39	2.39	0.10

Table 4
EVOLUTION OF THE MAJOR CANNABINOIDS CONTENT DURING STORAGE IN THE NATURAL LIGHT OF LABORATORY AT 22°C

In all samples the CBD content decreases as storage time increases, and the decrease is more pronounced for the samples exposed to light at 22°C. Although it is not experimentally confirmed, the CBD could undergo cyclization reaction to THC under some conditions (existence of enzyme CBD-cyclase which catalyzes the synthesis of THC via CBD), and this may explain its evolution during storage.

A pseudo first-order kinetic was used in order to calculate the kinetic parameters of the Δ^9-THC degradation such as rate constant (k), half-time ($t_{1/2}$), and the initial rate (v_0) under these storage conditions. As it can be seen from table 5, all the parameter values, excepting $t_{1/2}$, are always higher for the samples stored in the darkness at 4°C. These results suggest a higher rate of Δ^9-THC degradation in the herbal cannabis (a higer instability) in the normal storage conditions (natural light and ambiental temperature) than in the case of special storage conditions (darkness and low temperature).

On the other hand, the half-time parameter ($t_{1/2}$) characterizes the samples reactivity or the response strength of Δ^9-THC to environmental factors. This response is a measure of the sample stability and may be considered a new criterion for accounting the sample vigor and chemical potency. For example, the sample C1 potency might be considered higher than C2 sample potency, because its content in Δ^9-THC is higher (table 1). But, when

Region	Storage conditions					
	4°C, darkness			*22°C, natural light of laboratory*		
	k, months^{-1}	$t_{1/2}$, months	v_0, %/month	k, months^{-1}	$t_{1/2}$, months	v_0, %/month
C1	0.043	16.12	14.79	0.051	13.59	18.45
C2	0.034	20.39	6.18	0.042	16.50	6.52
C3	0.023	30.14	2.37	0.030	23.10	2.62
C4	0.017	40.78	1.44	0.023	30.14	1.61
C5	0.026	26.66	1.30	0.030	23.10	1.36
C6	0.029	23.90	1.26	0.037	18.73	1.44
C7	0.028	24.75	1.07	0.032	21.66	1.08
C8	0.012	57.76	0.036	0.025	27.72	0.057
C9	0.025	27.72	0.035	0.038	18.24	0.044
C10	0.039	17.78	0.0015	0.048	14.44	0.0029

Table 5
KINETIC PARAMETERS OF Δ^9-THC DEGRADATION CALCULATED FROM A PSEUDO FIRST ORDER KINETIC

the half-time parameter is taken as criterion in assessment of the sample chemical potency, it can be seen the samples C2-C4 longer half-times are presuming extended periods of storage at larger Δ^9-THC concentrations than the sample C1 (tables 3-5 and figures 4-6). Thus the new criterion brings about some different clues for ranking chemical potency and the real damage these drugs could perpetrate. More than that, this new criterion could be used for computing the approximate age of the samples. Nevertheless, some more accurate equations to describe the kinetics of the Δ^9-THC degradation and more accurate experiments for collecting real kinetic data are highly required for enforcing to this criterion as much strength as the Δ^9-THC concentration criterion has.

Conclusions

The chemical characterization of the cannabis plants from the ten different regions revealed their predominantly psychoactive character. In this respect, the experimental results showed that 70% of all samples (C1-C7) could be classified as drug-type, 20% as intermediate-type (C8 and C9) and 10% as fiber-type (C10).

During the storage of herbal cannabis, the content of its major cannabinoids varies depending on storage conditions. Thus, the degradation of Δ^9-THC is faster in the first year than in subsequent years. Furthermore, the degradation of Δ^9-THC in the samples exposed to light at 22°C is more advanced than in the samples stored in the darkness at 4°C. These results are consistent with those obtained from the kinetic study. In this respect, the calculated values of the kinetic parameters based on a pseudo first-order kinetic suggest a higher rate of Δ^9-THC degradation under normal storage conditions (natural light and ambient temperature) than under the special designed ones (darkness and low temperature).

The content of CBN increases during the storage, and the increase rates are higher in the samples exposed to light at 22°C than in those stored in the darkness at 4°C. These results are consistent with the variation of Δ^9-THC decay during decomposition process.

The CBD content decreases during storage especially for samples exposed to light at 22°C. This evolution could be explained by a cyclization reaction of CBD to Δ^9-THC in the presumed presence of the CBD-cyclase enzyme.

A new criterion for ranking chemical potency and the real damage these drugs are carrying out was suggested on the basis of kinetic parameters evaluated for a presumed pseudo first-order reaction of Δ^9-THC to CBN.

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