The Influence of Long-term Storage Conditions on the Stability of Cannabinoids derived from Cannabis Resin

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The objective of this paper was to investigate the changes in chemical potency of cannabis resin depending on its long-term storage conditions. In this respect, the content of tetrahydrocannabinol (Δ^9–THC), cannabinol (CBN), and cannabidiol (CBD) in cannabis resin derived from three different seizures made by criminal prosecution authorities from Romania were measured for up to four years of storage in darkness at 4°C and in laboratory light at 22°C. The results revealed a steadily decay of Δ^9–THC over the entire storage period. In addition, the samples exhibited a more pronounced decay for the sample exposed to light at 22°C than those stored in darkness at 4°C. For CBD decay, the same trend is valid also. On the contrary, the content of CBN raised steadily during storage, and the raise is more pronounced for the samples exposed to light at 22°C than those stored in the darkness at 4°C.

Keywords: cannabis, resin, potency, cannabinoid

Cannabis resin, known as hashish, is a cannabis product with high potency, produced mostly from resinous secretions of the female flowering tops glandular trichomes of the cannabis plants [1]. It is well known that potency of the cannabis products including cannabis resin depend on the tetrahydrocannabinol or Δ^9–THC content [2]. This psychoactive cannabinoid has a different stability in various environmental conditions [3-5]. Lindholst [6] has shown that the cannabinoid stability in cannabis products, including cannabis resin is influenced by light, temperature and oxygen availability. It addition, it has been demonstrated the potency of herbal cannabis decreases as its storage time increases. Moreover, the daylight and normal temperatures increase the degradation processes of both Δ^9–THC and CBD (cannabidinol) and subsequently increase the formation degree of CBN (cannabinol) [7]. While the degradation of Δ^9–THC to CBN seems to be of more chemical nature [8], the degradation of CBD seems to be of more biochemical nature, one of the possible degradation routs being by catalyzed (CBD-cyclase) cyclization [9].

Although the cannabis products were intensively studied, it is imperative to further explore then, in order to get more insights concerning their behaviour under different environmental conditions. Therefore, the aim of this paper is to experimentally investigate the content of the major cannabinoids versus time storage and the influence of storage conditions such as temperature and light on the chemical potency of cannabis resin.

Experimental part

Materials and methods

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

Cannabis resin samples

Cannabis resin from three different seizures (marked with symbols from R1 to R3) made by criminal prosecution authorities from Romania and provided by Central Laboratory for Drug Analysis and Profiling were subject to experimental investigation. At the time of their seizure, the samples found were in form of brown tablets as it is shown in the figure 1. The brown color of the tablets is due to oxidation processes expanding over outer layer only. The remaining material from inner layer of the tablets is green-olive. In this respect, the samples subject to experimental investigation were collected from inner layer, in order to avoid staining with the oxidized material. The samples were stored either in the darkness at 4°C and in the laboratory light at 22°C for four years. At regular intervals (every three months), samples were taken for analysis in order to determine the content of their major cannabinoids, namely Δ^9–THC, CBD, and CBN.

The procedure used for sample preparation consisted of extracting 0.1 g of cannabis resin with 20 mL of a methanol-chloroform (9:1, v/v) mixture. Each sample was shaken for 30 min and then placed in an ultrasonic bath at ambient temperature for 15 min for missing the efficiency in cannabinoids of interest. The extract was filtered and an aliquot (0.6 mL) of the 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 when the traces of tetrahydrocannabinolic acid (THCA) are decarboxylated. Decarboxylation is highly required when the entire content of Δ^9–THC of the sample has to be measured. Before analyses, the residue was extracted in 1.5 mL extraction solvent (methanol-chloroform 9:1, v/v).

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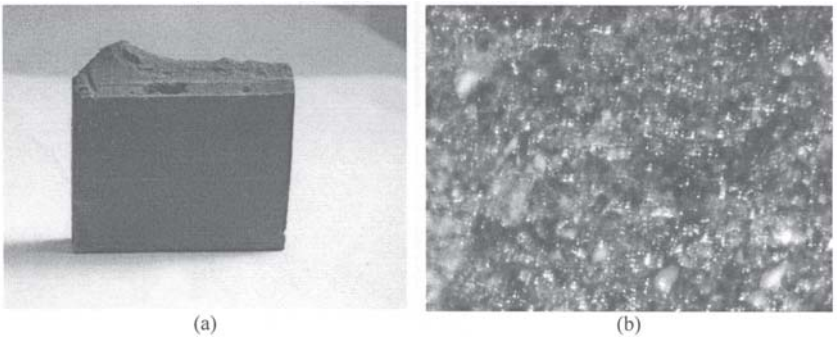


Fig.1 Macroscopic (a) and microscopic (b) pictures of cannabis resin

After this, the sample was subject to analyses for the determination of the the major cannabinoids content (Δ^9–THC, CBD, and CBN) [10].

Analytical protocol

Extracts obtained by procedure described above have been subject to analytical investigations through instrumental methods (GC-FID – Gas Chromatography–Flame Ionization Detector and HPLC – High-Performance Liquid Chromatography).

GC-FID analyses were carried out on a 7890A gas chromatograph with a flame ionization detector (Agilent, Waldbronn, Germany). Separation was achieved on a fused silica capillary column (HP–5MS, 30 m×0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Temperature program: 150°C hold for 1 min, 10°C/min to 280°C, hold for 5 min. The injection port and interface temperature were 250 and 300°C, respectively. Split injection mode was used (20:1) and hydrogen, with a flow rate of 30 mL per min, was used as carrier gas [11].

HPLC analyses were carried out on an Agilent 1100 Series HPLC chromatograph (Agilent, Waldbronn, Germany) equipped with a quaternary pump, autosempler, 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 [12].

Results and discussions

Based on the above samples preparation procedure and both GC-FID and HPLC analysis the content of the cannabinoids of interest in cannabis resin were determined. The experimental results are presented in table 1. Regarding the content of their major cannabinoids, there are no large differences between the three samples as it can be seen from the table 1. Moreover, in all samples the content of CBN is very low. These results suggest that the degradation processes are taking place in early stages and the degree of freshness is the same in the all the samples. Thus, the sampled material is ready to be used for meaningful comparative purposes in the following experiments.

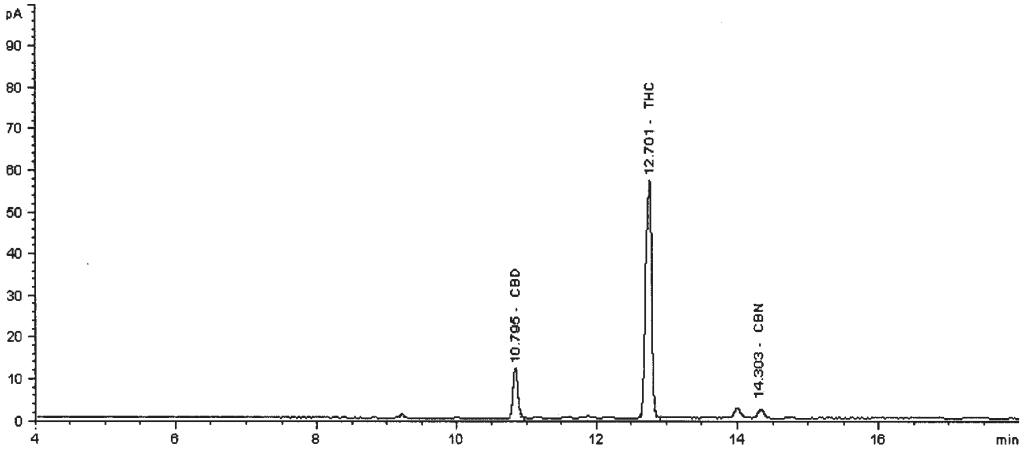
The experimental results concerning the stability of the major cannabinoids indicate a small but constant difference between cannabinoids content of the cannabis resin as a function of storage conditions. Figures 2 and 3 show the GC-FID and HPLC chromatograms corresponding to cannabis resin derived from seizure R1 after one year of storage in different conditions. Data provided by these chromatograms outlines that the decay degree of Δ^9–THC in the first year of storage of the samples in the laboratory light at 22°C is about 1.02 times higher than that recorded for samples stored in the darkness at 4°C. At the same time, the formation degree of CBN in the same year in the samples exposed to laboratory light at 22°C is about 1.10 times higher than that recorded for samples stored in the darkness at 4°C. On the contrary, the decay degree of CBD in the first year of storage of the samples in the laboratory light at 22°C is about 0.62 times smaller than that recorded for samples stored in the darkness at 4°C.

Table 1
THE INITIAL CONTENT OF MAJOR CANNABINOIDS

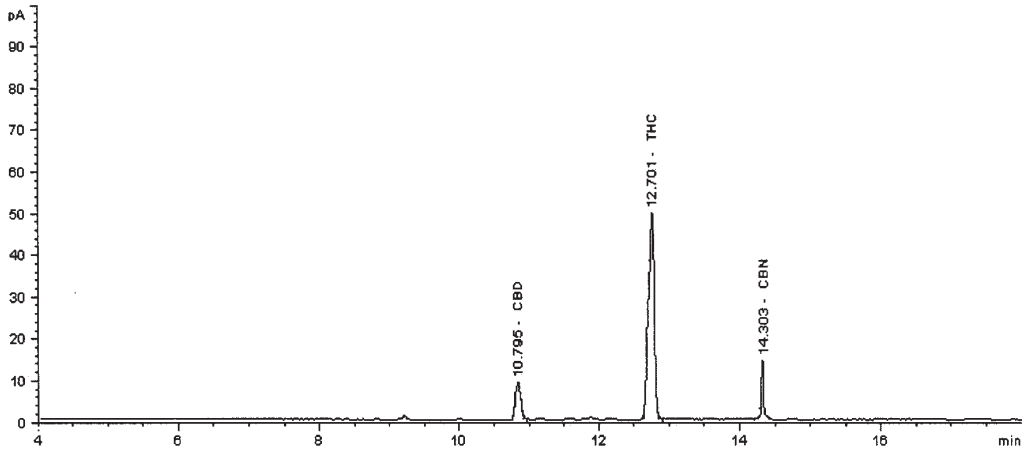
Cannabinoid, %	Seizure		
	R1	*R2*	*R3*
Δ^9-THC	21.96	17.88	23.90
CBN	0.63	0.72	0.81
CBD	4.61	5.12	5.84

Figures 4–6 show the variation of the major cannabinoids content in all samples of cannabis resin as a function of time and storage conditions. In all causes the Δ^9–THC content decreases during storage and is always higher in the samples stored in the darkness at 4°C than in the samples stored in the laboratory light. The same trend is suggested by the CBD content variation. In contrast with these results, the CBN content increases during storage and is always higher in the samples stored in laboratory light at 22°C than the samples stored in darkness at 4°C. The results, concerning all samples from all seizures time evolution of the major cannabinoids content during storage in different conditions, are given in the table 2.

The results highlighted a steadily decay of Δ^9–THC over the entire storage period up to a very low content. Moreover, 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 seizure R1 stored in the darkness at 4°C, 25.22% of Δ^9–THC (fig. 4 a) was lost in the first year with an average loss of 6.30% every tree months, 25.14% in the second year with an average loss of 6.29, 24.91% in the third year with an average loss of 6.23 and 20.49% in the fourth year with an average loss of 5.12%. In the case of samples from the same seizure, but stored in the laboratory light at 22°C, 25.66% of Δ^9–THC was lost in the first year with an average loss of 6.42% every tree months, 25.85% in the second year with an average loss of 6.46, 25.46% in the third year with an average loss of 6.37 and 20.20% in the fourth year with an average loss of 5.05%. Finally, after four years, the samples stored in the darkness at 4°C lost 95.77% of Δ^9–THC and the samples stored in the laboratory light at 22°C lost 97.18% of Δ^9–THC (with 1.41% more). Regarding the variation of CBN (fig. 4 b) corresponding to the same seizure over the storage period, the experimental results highlighted the following features: samples stored in the darkness at 4°C, 59.19% of CBN was formed in the first year with an average gain of 14.80% every three months, 19.45% in the second year with an average gain of 4.86, 5.40% in the third year with an average gain of 1.35%, and 4.96% in the fourth year with an average gain of 1.24%; samples from the same seizure but stored in the laboratory



(a)



(b)

Fig. 2 GC–FID chromatograms of cannabis resin (R1) stored in darkness at 4°C (a) and in laboratory light at 22°C (b)

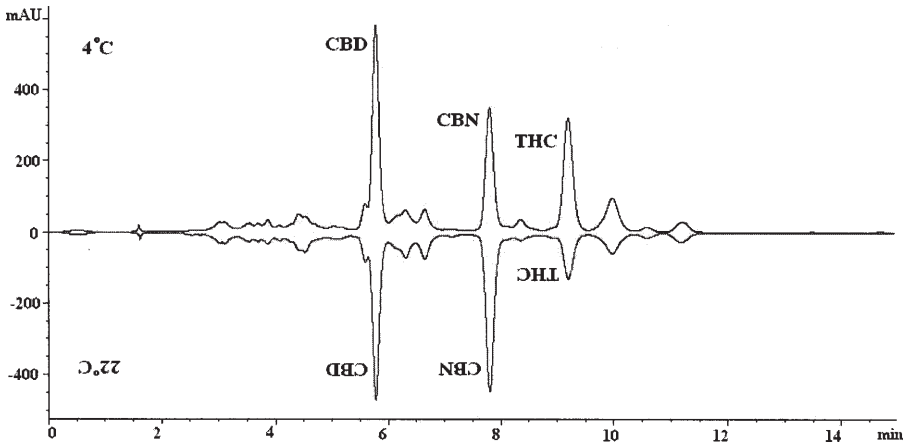
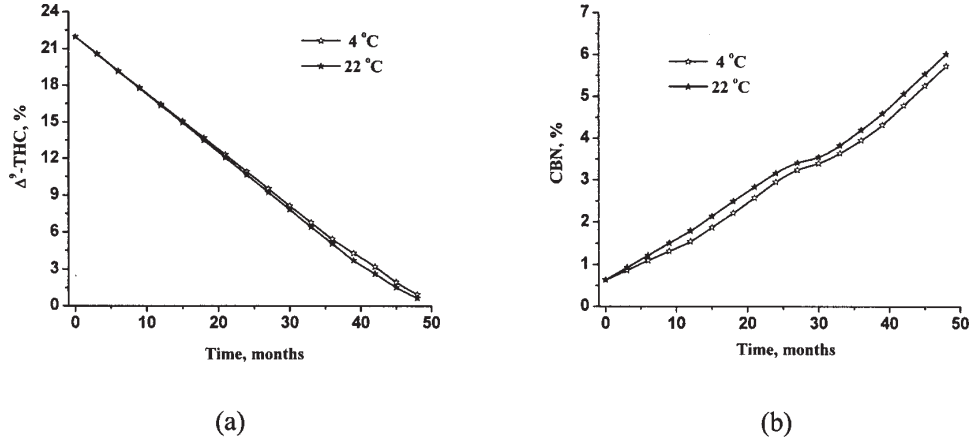


Fig. 3 HPLC chromatograms of cannabis resin stored in darkness at 4°C (upper side) and in laboratory light at 22°C (down side)

light at 22°C, 64.88% of CBN was formed in the first year with an average gain of 16.22% every tree months, 15.21% in the second year with an average gain of 3.88, 4.89% in the third year with an average gain of 6.37 and 4.56% in the fourth year with an average gain of 1.14%. Finally, after four years, the samples stored in the darkness at 4°C gained 89% of CBN and the samples stored in the laboratory light at 22°C gained 89.54% of CBN (with 0.54% more). The same trend was recorded for all cannabis resin samples.

Comparing the decay degree of Δ^9–THC in the first year of storage period of cannabis resin with the formation degree of CBN in the same year, it can be seen that the latter is with 40% higher than the first. Thus, the changes regarding the content of CBN during the storage period can not be put solely on chemical and/or biochemical conversion processes of Δ^9–THC into CBN. Thus, other degrading routs of other cannabinolic compounds must be considered as contributors to the overall increase of CBN content upon long-term storage.

Regarding the variation of CBD (fig. 4 c) corresponding to the same seizure over the storage period, the experimental results highlighted follows. In the case of samples from seizure R1 stored in the darkness at 4°C, 21.07% of CBD was lost in the first year with an average loss of 5.27% every tree months, 9.67% in the second year with an average loss of 2.42, 10.66% in the third year with an average loss of 2.66 and 14.35% in the fourth year with an average loss of 3.59%; samples from the same seizure but stored in the laboratory light at 22°C, 12.96% of CBD was lost in the first year with an average loss of 3.24% every three months, 13.24% in the second year with an average loss of 3.31, 8.77% in the third year with an average loss of 2.19 and 9.92% in the fourth year with an average



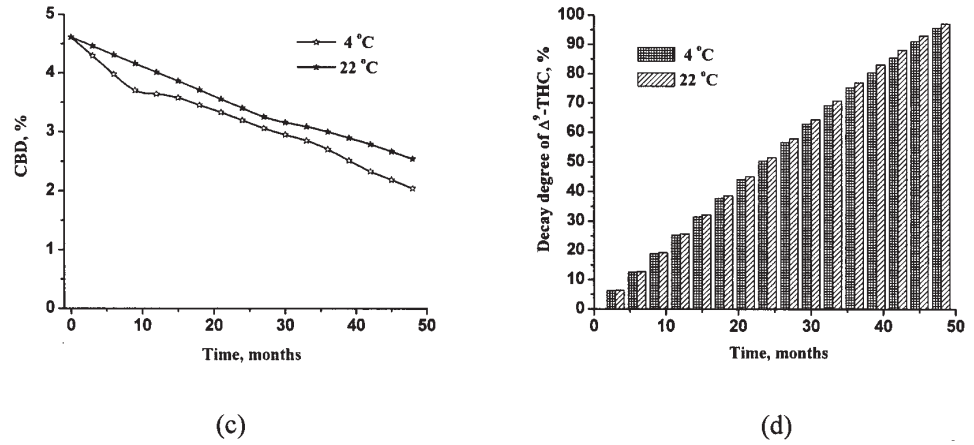
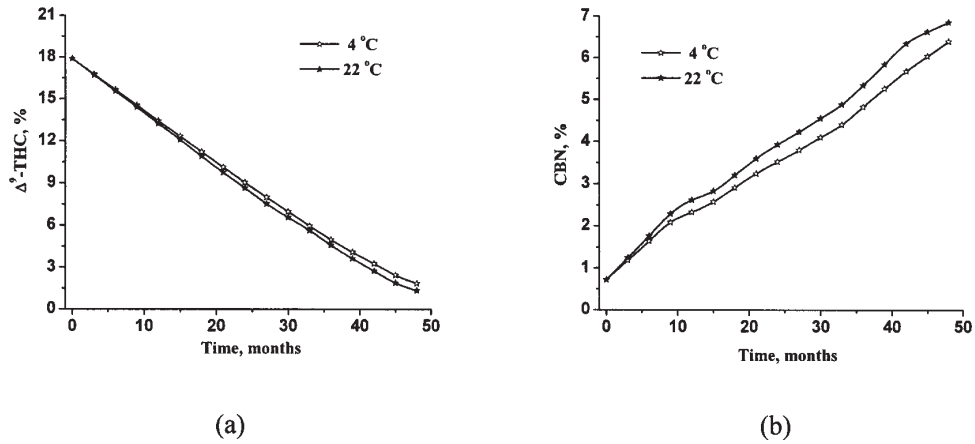


Fig. 4 Variation of cannabinoids content in cannabis resin derived from seizure R1: (a) Δ^9–THC decay; (b) CBN formation; (c) CBD decay; (d) decay degree of Δ^9–THC



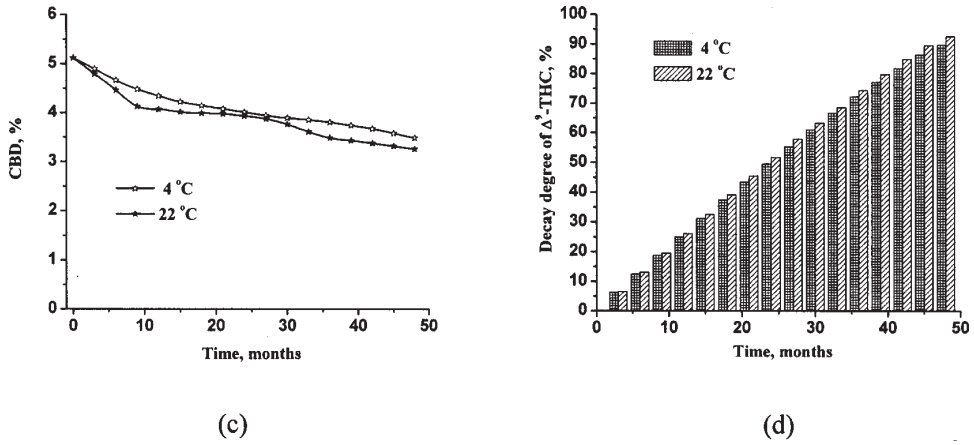


Fig. 5 Variation of cannabinoids content in cannabis resin derived from seizure R2: (a) Δ^9–THC decay; (b) CBN formation; (c) CBD decay; (d) decay degree of Δ^9–THC

loss of 2.48%. Finally, after four years, the samples stored in the darkness at 4°C lost 55.75% of CBD and the samples stored in the laboratory light at 22°C lost 44.89% of CBD (with less 10.86%). The same trend was recorded for the rest of cannabis resin samples.

Analyzing these results it can be seen that the decay degree of CBD in the first year of storage period of cannabis resin is about 17% in the case of the samples stored in the darkness at 4°C and about 19% in the case of the samples stored in the laboratory light at 22°C. These values are

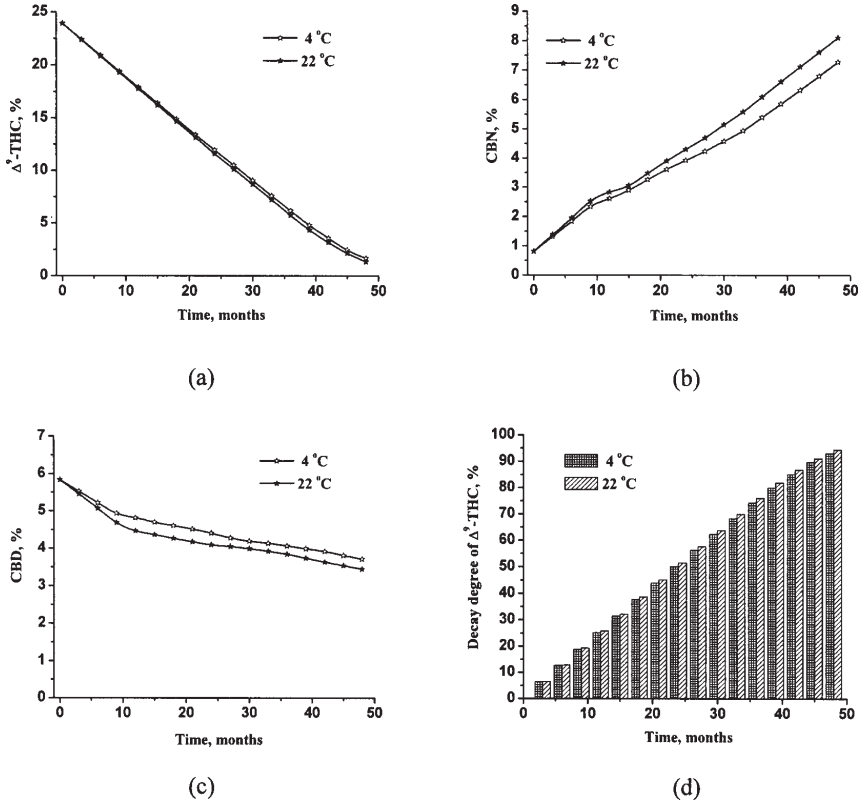


Fig. 6 Variation of cannabinoids content in cannabis resin derived from seizure R3: (a)Δ^9–THC decay; (b) CBN formation; (c) CBD decay; (d) decay degree of Δ^9–THC

Cannabinoid %	Time years	Seizure					
		R1		R2		R3	
		darkness, 4 °C	*light, 22 °C*	*darkness, 4 °C*	*light, 22 °C*	*darkness, 4 °C*	*light, 22 °C*
Δ^9–THC	1	16.42	16.32	13.42	13.22	17.89	17.74
	2	10.90	10.65	9.04	8.64	11.93	11.58
	3	5.43	5.06	4.97	4.59	6.15	5.72
	4	0.93	0.62	1.85	1.33	1.67	1.32
CBN	1	1.54	1.79	2.33	2.61	2.61	2.83
	2	2.95	3.16	3.52	3.93	3.92	4.30
	3	3.95	4.19	4.83	5.34	5.40	6.10
	4	5.73	6.02	6.40	6.85	7.28	8.11
CBD	1	3.64	4.01	4.34	4.07	4.81	4.46
	2	3.19	3.40	4.01	3.93	4.40	4.09
	3	2.70	3.00	3.80	3.48	4.07	3.84
	4	2.09	2.54	3.48	3.25	3.71	3.44

Table 2
EVOLUTION OF THE MAJOR CANNABINOIDS CONTENT DURING STORAGE IN DIFFERENT CONDITIONS

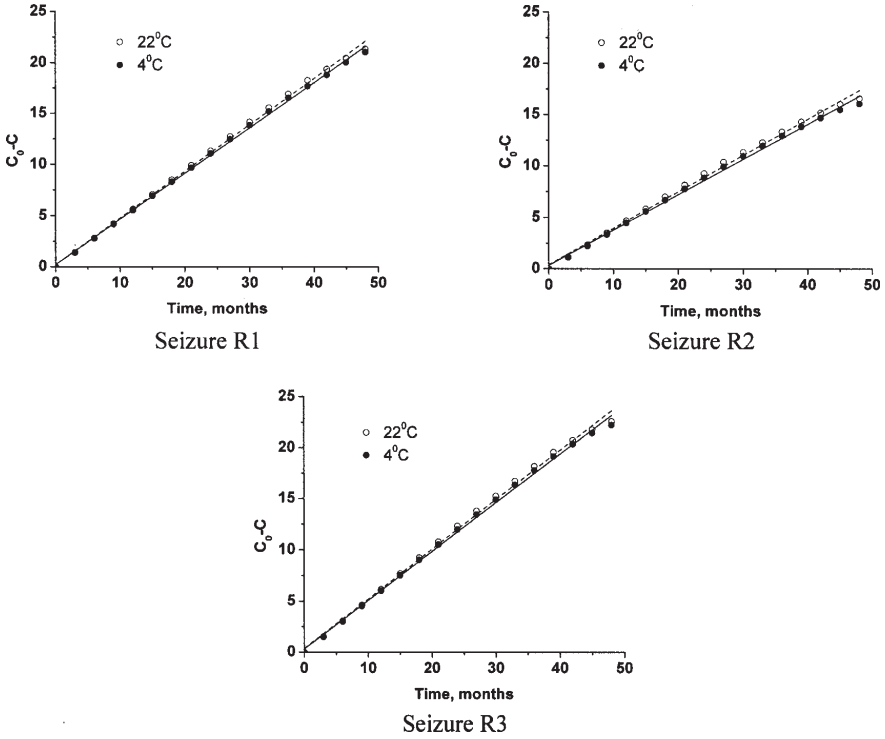


Fig. 7. Pseudo zero-order kinetic of Δ^9–THC decay in the cannabis resin; the solid line represents the linear regression of data corresponding to 4°C and darkness storage conditions and, the dashed line represents the linear regression of data corresponding to 22°C and laboratory light storage conditions

Table 3
KINETIC PARAMETERS OF Δ^9-THC DECAY CALCULATED

Seizure	Storage conditions					
	4°C, darkness			*22°C, laboratory light*		
	k', months^{-1}	$t_{1/2}$, months	v, %/month	k', months^{-1}	$t_{1/2}$, months	v, %/month
R1	0.44	24.95	9.66	0.45	24.40	9.88
R2	0.34	26.29	6.09	0.35	25.54	6.26
R3	0.48	24.9	11.47	0.48	24.89	11.47

approximately half the difference of 40% between the decay degree of Δ^9–THC and the formation degree of CBN. Hence, if the cyclization of CBD to Δ^9–THC and further Δ^9–THC decay of to CBN are accepted, the changes regarding the content of CBN during the storage period could be justified.

A pseudo zero-order kinetic was used (fig. 7) in order to calculate the kinetic parameters of the Δ^9–THC decay such as the rate constant (k'), the half–time ($t_{1/2}$), and the decay rate (v) in these storage conditions. As it can be seen from table 3, both rate constant and decay rate are higher (except for samples derived from seizure R3 when the values are the same) in the samples stored in the laboratory light at 22°C than those stored in the darkness at 4°C. On the contrary, the values of the half-time corresponding to the samples stored in the laboratory light at 22°C are smaller than those dark stored samples at 4°C. These results suggest a higher rate of Δ^9–THC decay in the cannabis resin in the normal storage conditions (natural light and ambiental temperature) than in the case of special storage conditions (darkness and low temperature).

Conclusions

The experimental results regarding the stability of the major cannabinoids highlighted a small but constant difference between cannabinoids content of the cannabis resin as a function of storage conditions. Thus, the results revealed a steadily decay of Δ^9–THC over the entire storage period up to a very low content. Moreover, the decay of Δ^9–THC contained in the samples exposed to light at 22°C is a more pronounced one than in the samples stored in the darkness at 4°C. The content of CBN increases during storage and increase is, also, 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. The CBD content decreases during storage, especially for samples exposed to light at 22°C. This evolution could be justified, only if the cyclization of CBD to Δ^9–THC in the presence of postulated CBD–cyclase enzyme and further decay of Δ^9–THC to CBN are accepted as a degrading route.

The decay of Δ^9–THC takes place up on a pseudo zero-order kinetic and the calculated values of the kinetic parameters suggest a little higher rate of Δ^9–THC degradation in normal storage conditions namely natural light and ambiental temperature than in special ones namely darkness and low temperature.

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