INFLUENCE OF TEMPERATURE AND HEATING TIME ON THE DECARBOXYLATION OF Δ^9-THCA AND CBDA IN THE CANNABIS INFLORESCENCES

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The influence of temperature and heating time on the decarboxylation of Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) and cannabidiolic acid (CBDA) of some seized cannabis inflorescences was studied in this work. The results showed that the decarboxylation rate increases with increasing temperature, especially in the temperature range between 75 and 150 °C for Δ^9-THCA, respectively 100 and 175 °C for CBDA. The maximum value for Δ^9-THC recorded throughout the temperature range at which the experiments were performed was found to be approximately constant. This result suggests a nearly complete conversion of Δ^9-THCA to Δ^9-THC. The decarboxylation of CBDA does not seem to proceed completely to CBD, the results obtained indicating the possibility that some secondary compounds may also be the product of this transformation.

Keywords: cannabis inflorescences, acid cannabinoids, decarboxylation

1. Introduction

The cannabinoids are a unique class of C21 terpenophenolic compounds that are found in the cannabis plants [1]. They are biosynthesized through enzymatic catalysis of some specific reactions which take place in specialized gland cells of the plant called trichomes, which are present predominantly in the inflorescences [2]. The most abundant cannabinoids are Δ^9-tetrahydrocannabinol (Δ^9-THC) which is the main psychoactive cannabinoid, and canabidiol (CBD) which is one of the most important pharmacologically active cannabinoid [3].

Cannabinoids are initially biosynthesized as carboxylic acids (Δ^9-tetrahydrocannabinolic acid – Δ^9-THCA, cannabidiolic acid – CBDA, etc.) from the natural precursor cannabigerolic acid (CBGA), which subjected to heating, they are decarboxylated being converted to their neutral forms (Δ^9-THC, CBD, etc.) [4]. A simplified schematic representation of the occurring reactions are shown in Fig. 1. The exposure to light, heating or even the simple aging process

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of cannabis plants lead to some oxidative processes, Δ^9-THC being predominantly converted to cannabinol (CBN) [5], and CBD undergoing a molecular rearrangements or participating in dimerization or trimerization reactions [6].

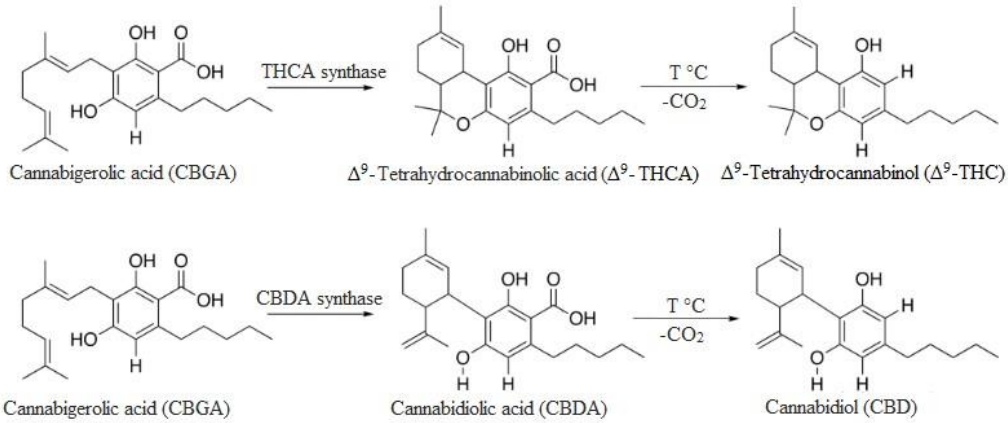


Fig. 1. Biosynthesis of cannabinoid carboxylic acids and their decarboxylation

The decarboxylation process of acidic cannabinoids is important from both analytical and forensic point of view. In this respect, from analytical point of view, consistent efforts are being made to identify the best analytical ways to determine, as accurately as possible, the total content of neutral cannabinoids (derived mainly from their carboxylic precursors) in the cannabis plants. This involves the optimization of the sample preparation procedures (sampling, drying, grinding, homogenization, etc.), followed by the preliminary stages of sample processing (extraction, solvent removal, decarboxylation, etc.), and the actual analysis methods (gas chromatography, liquid chromatography, etc.) [7 – 10]. From the forensic point of view, it is desired to establish as accurately and quickly as possible, the chemical potency/strength of the cannabis seizures, and to identify their chemotype. The total Δ^9-THC content of seizers, which usually derives from the decarboxylation of Δ^9-THCA, represents their chemical potency [11 – 14], and establishing of the actual Δ^9-THCA/CBDA ratio helps to identify the chemotype of young cannabis plants, particularly important from forensic standpoint, as it is no longer necessary to expect their flowering stage [15].

The study of decarboxylation process of acidic cannabinoids involves the analysis of the main factors that may influence the process, such as the type of sample, the representativeness of the sample, the sample homogeneity, storage and preparation procedures, the operating decarboxylation conditions such as temperature, pressure, analytical technique used, etc. Among these specific working conditions, the temperature and heating time seems to have a high influence on the degree of decarboxylation, as well as on the decarboxylation mechanisms [16]. The decarboxylation process has also been studied under

different working conditions, generating either similar or contradictory results, namely in open [17] or closed reactors [18], on a glass surface of the reactor or on the surface of various adsorbents packed beds reactors or in different solvents media [19]. Therefore, more research in this area is imperatively needed in order to clarify these issues.

The objective of this paper, generated by the above presented challenges, is to investigate the influence of temperature and heating time on the decarboxylation of Δ^9-THCA and CBDA, allowing for a better backtrack analysis and estimation of the active compounds in the seized cannabis inflorescences, even after a certain storage time, situation commonly encountered in the actual capturing and legally enforced procedures.

2. Experimental

2.1. Materials

Cannabis inflorescences (the whole flower heads) seized by Romanian law enforcement authorities were used in the experiments carried out in the Central Laboratory for Drug Analysis and Profiling of Bucharest, Romania. Analytical grade methanol (Merck, Darmstadt, Germany) was used as solvent throughout the experiments. The standard solutions used in the analysis of cannabinoids contained in the cannabis inflorescences extracts, namely delta-9-tetrahydrocannabinol (Δ^9-THC), cannabinol (CBN), and cannabidiol (CBD) were purchased from Lipomed, Arlesheim, Switzerland.

2.2. Thermal decarboxylation experiments

The cannabis inflorescences subjected to analysis were dried, homogenized, and finely milled up to a particle size diameter smaller than 75 μm. Aliquots of 200 mg of cannabis inflorescences powder were extracted with 20 mL of methanol for 20 minutes into brown glass vials, placed into an ultrasonic bath. The obtained extracts were filtered, transferred to new glass vials and placed into a thermostatic chamber at 50 °C in order to remove the solvent. Prior to this, a corresponding number of samples were taken from the filtered extracts to determine the initial cannabinoids content. The residues remaining after the solvent evaporation were further subjected to thermal decarboxylation, the open vials containing the residues were heated in an oven at temperatures of 75 °C and 100 °C for heating times ranging between 2 and 900 minutes, 125 °C and 150 °C for a heating time ranging between 2 and 600 minutes, and 175 °C, 200 °C, and 220°C for a heating time ranging between 2 and 180 minutes. The cannabinoids content was determined at every two minutes in the range of 0 – 30 minutes, at every ten minutes in the range of 30 – 100 minutes, and at every twenty minutes in the range of 100 – 900 minutes. At the end of each heating time, the residues

were re-dissolved in methanol and the solutions obtained were filtered and prepared for analysis in order to determine the cannabinoids content.

2.3. Cannabinoids analysis

High-performance liquid chromatography (HPLC) with a diode-array detector (DAD) was used in this work to quantitative analysis of cannabinoids. The analyzes were performed on an Agilent 1100 Series HPLC chromatograph (Agilent, Waldbronn, Germany) equipped with a quaternary pump, auto-sampler, chromatographic column oven, and diode-array detector (DAD) UV Lamp ON (223 nm). The separation was carried out on a Hypersil ODS chromatographic column with the following characteristics: 250 mm (length) × 4.6 mm (internal diameter), 5 µm stationary phase thickness. The equipment operates with a constant flow of 1 mL mobile phase/minute. The mobile phase was acetonitrile 80% and 20% ultrapure water. The concentration of the cannabinoids in the samples was determined based on the calibration curves shown in Fig. 2 made for each cannabinoid separately by using the corresponding standard solutions.

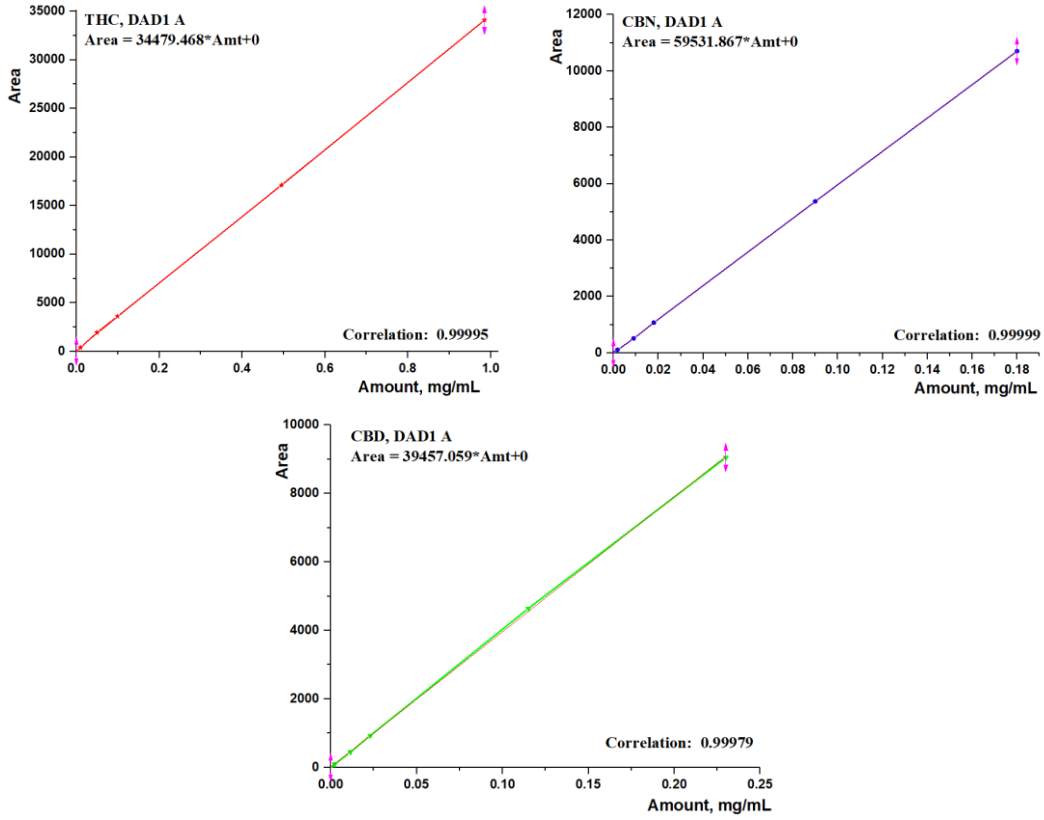


Fig. 2. Calibration curves for Δ^9-THC, CBN, and CBD

The correlation coefficients of the calibration (expressed as coefficient of determination, R^2) derived from the linear regression highlighted a linear variation

(integrated area of the peaks vs concentration of cannabinoids) over the concentration range of 10 – 1000 mg/L for Δ^9-THC (R^2 = 0.99995), 2 – 180 mg/L for CBN (R^2 = 0.99999), and 2 – 230 mg/L for CBD (R^2 = 0.99979). A typical HPLC chromatogram is shown in Fig.3.

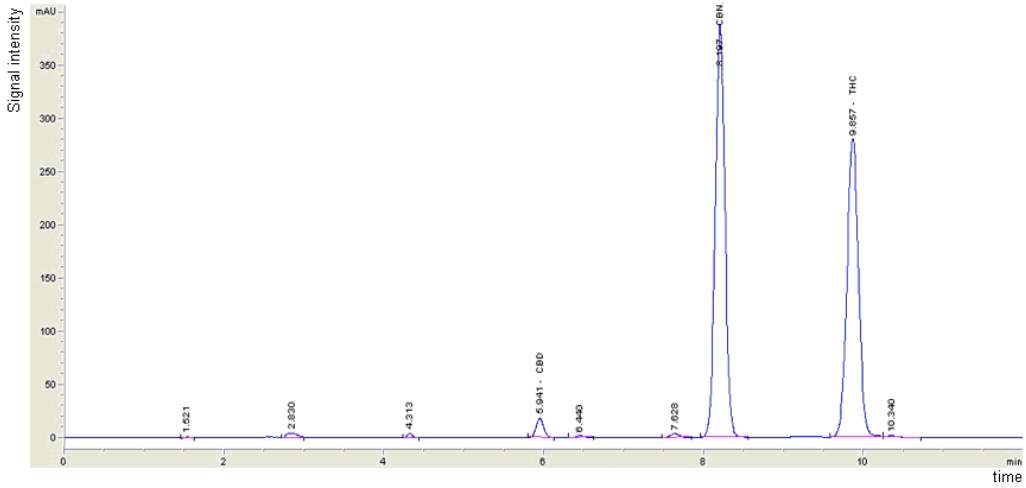
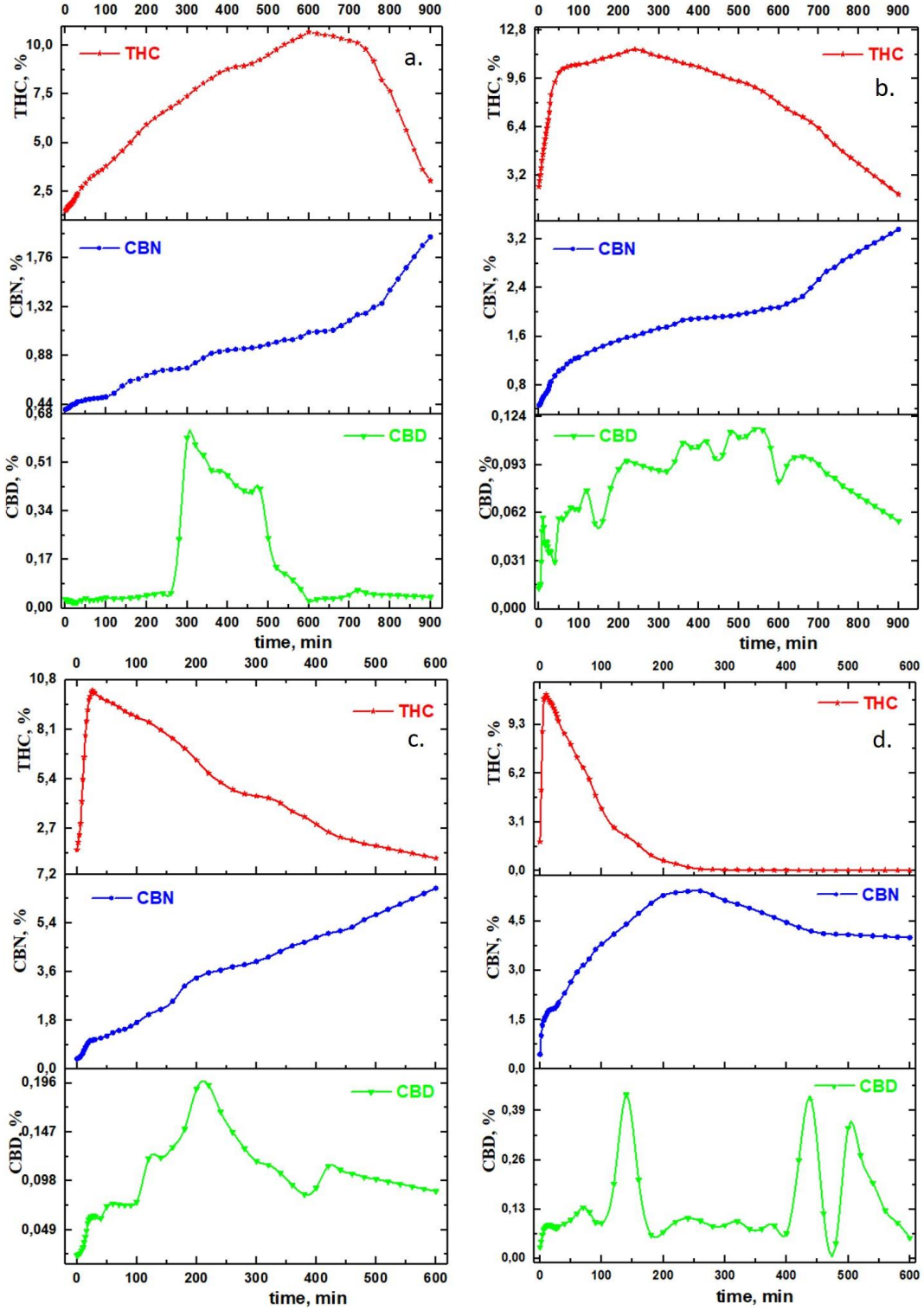


Fig. 3. HPLC chromatogram for quantitative analysis of cannabinoids

3. Results and Discussion

HPLC-DAD analysis showed that the initial average content of cannabinoids in the samples (% dry weight – g of cannabinoid/100 g of dried cannabis inflorescences) is of 1.92 % Δ^9-THC, 0.44% CBN, and 0.04 % CBD. The low Δ^9-THC initial content in the samples denotes a low degree of decarboxylation of Δ^9-THCA (delta-9-tetrahydrocannabinolic acid), which suggests that the seized cannabis inflorescences are relatively fresh and have been kept under optimal conditions. These findings are also supported by the low initial content of CBN in the samples which is closely related to the Δ^9-THC content, the CBN being the major degradation product of Δ^9-THC by the action of heat and light [20]. The low decarboxylation degree of CBDA (cannabidiolic acid), originally present in the cannabis inflorescences, is also highlighted by the low initial content of CBD in the samples. The variation of the cannabinoids content in the samples subjected to the thermal decarboxylation is shown in Fig. 4. As one can see in figure 4a, the rate of the decarboxylation process of the Δ^9-THCA at 75 °C is very slow, the maximum concentration of Δ^9-THC (10.69 % w/w) being obtained after 600 minutes of heating. The CBN content of the samples increased steadily with increasing heating time from 0 to 780 minutes (an increase of 1.00 % over 13 hours).


a.
b.
c.
d.
THC
CBN
CBD
THC, %
CBN, %
CBD, %
time, min

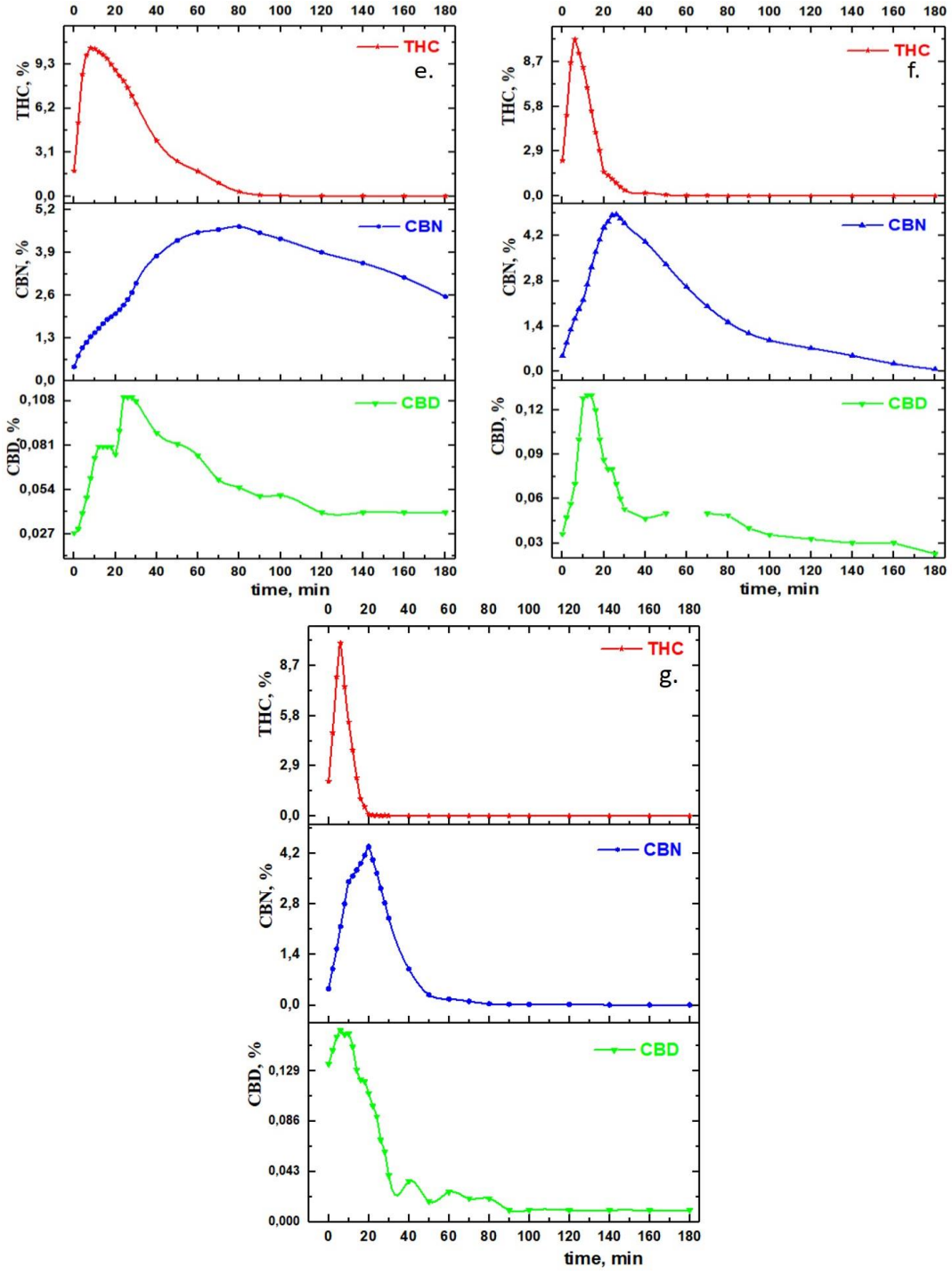


Fig. 4. Variation of the cannabinoids content of the samples according to the decarboxylation temperature and the heating time of the samples: a. 75 °C; b. 100 °C; c. 125 °C; d, 150 °C; e. 175 °C; f. 200 °C; g. 225 °C

After this, a marked increase has been recorded (an increase of 0.60 % in 2 hours) due to the increasing rate of the decarboxylation process of the Δ^9-THCA in the samples and also due to the beginning of a massive degradation of the Δ^9-THC derived from the decarboxylation of Δ^9-THCA. Regarding the evolution of the CBD content it can be observed a sudden increase after 280 minutes of heating (the maximum content of CBD recorded at 300 minutes is twelve times higher compared to the one recorded at 260 minutes). After another 300 minutes the content of CBD decreased to the initial value. This result may be due to the slow decarboxylation of CBDA at this temperature in the first five hours of heating, followed by an increase of the decarboxylation rate during which, for 40 minutes, CBD accumulates rapidly. Subsequently, the content in CBD decreases either due to its degradation or its loss by vaporization.

The evolution of the decarboxylation process at 100 °C (Fig. 4b) is similar in terms of Δ^9-THC and CBN content with that recorded at 75 °C, the difference consisting of the maximum Δ^9-THC content and the heating time at which it was recorded as well as the heating time at which the marked increase in CBN content begins. Thus, the maximum Δ^9-THC content of 11.52 % was obtained at a heating time of 240 minutes, and a marked increase in the CBN content was recorded after 620 minutes. Regarding the evolution of CBD content, this is different from the one recorded at 75 °C in that, this time, there is an increase in CBD content since the beginning of heating at 100 °C. In this respect, the CBD content increases progressively reaching the maximum of 0.12 % after 540 minutes of heating.

At the heating temperature of 125 °C (Fig. 4c), there is a marked increase in Δ^9-THC content from the first few minutes of heating, the maximum of 10.23 % being recorded after 26 minutes of heating. Subsequently, the content in Δ^9-THC decreases almost linearly, the evolution being almost identical to that of the increase in CBN content. It is worth noting that the heating time at 125 °C at which the maximum Δ^9-THC content is recorded is approximately nine times lower than the value recorded at 100 °C. Regarding the content of CBD, it presents a maximum of 0.19 % at a heating time of 200 minutes, thus registering a remarkable progress regarding the decarboxylation rate of CBDA compared to the values recorded at lower temperatures.

Regarding the heating temperature of 150 °C (Fig. 4d), the heating time at which the maximum Δ^9-THC content of 11.20 % is recorded decreases 2.6 times compared to the value recorded at the heating temperature of 125 °C. Starting with this heating temperature, the CBN content has a maximum value (5.43 %), which corresponds to the heating time (260 °C) at which the Δ^9-THC content reaches the range of minimum values indicating its almost complete removing from the system. This might denote either that it begins to degrade, or it is lost by vaporization, especially that the heating temperature approaching its boiling

temperature (185 °C). The CBD content registers also a maximum value of 0.43 % at the heating time of 140 minutes, which is in a continuous decrease compared to the values recorded at lower temperatures.

The evolution of the cannabinoids content at the heating temperatures of 175 °C, 200 °C, and 225 °C, (Figs. 4e-g) is similar to the one recorded at the heating temperature of 150 °C, with the difference that the heating time at which the maximum content of cannabinoids is recorded slightly decreases as the temperature increases. Thus, in the case of Δ^9-THC the heating time at which the maximum content is reached is 8 minutes for a heating temperature of 175 °C (10.43 %), 6 minutes for 200 °C (10.16 %), and 6 minutes for 225 °C (10.02 %). In the case of CBN, the heating time corresponding to its maximum content is 80 minutes for 175 °C (4.69 %), 26 minutes for 200 °C (4.86 %), and 20 minutes for 225 °C (4.39 %). The heating time at which the maximum CBD is reached is 24 minute for 175 °C (0.11 %), 10 minutes for 200 °C (0.13 %), and 4 minutes for 225 °C (0.16 %).

It is worth noting that, starting with the temperature of 150 °C, the heating time from which the Δ^9-THC can be practically considered as completely removed from the system decreases considerably. Thus, it can be considered that at 150 °C the Δ^9-THC is completely removed from the system at a heating time of 240 minutes, which drops to 90 minutes at 175 °C, 50 minutes at 200 °C, and 20 minutes at 225 °C (Figs. 4d-g). At temperatures above 200 °C (Figs. 4e-g) there are similar trends for the other two cannabinoids studied, namely CBN and CBD. This could be mainly due to reaching or exceeding the boiling temperature of these cannabinoids, evaporation being the main way of removing them at temperatures higher than 175 °C.

Fig. 5 shows the variation of the maximum cannabinoids content as well as the heating time at which this maximum content is reached as a function of decarboxylation temperature, where a,b,c, represent the variation of the maximum content of Δ^9-THC, Fig. 5.a., the variation of the maximum content of CBN, Fig. 5.b., and the variation of the maximum content of CBD, Fig. 5.c. As can be seen, the heating time at which the maximum Δ^9-THC content is recorded decreases greatly in the temperature range 75 – 125 °C after which, the decrease becomes insignificant. However, it seems that the maximum content of Δ^9-THC does not change so much with the increase of the decarboxylation temperature, although during the temperature range 150 – 225 °C there is a slight decrease (Fig. 5a). This might suggest a complete decarboxylation of Δ^9-THCA to Δ^9-THC regardless of temperature, the small variations recorded most likely coming from errors associated with the experiments.

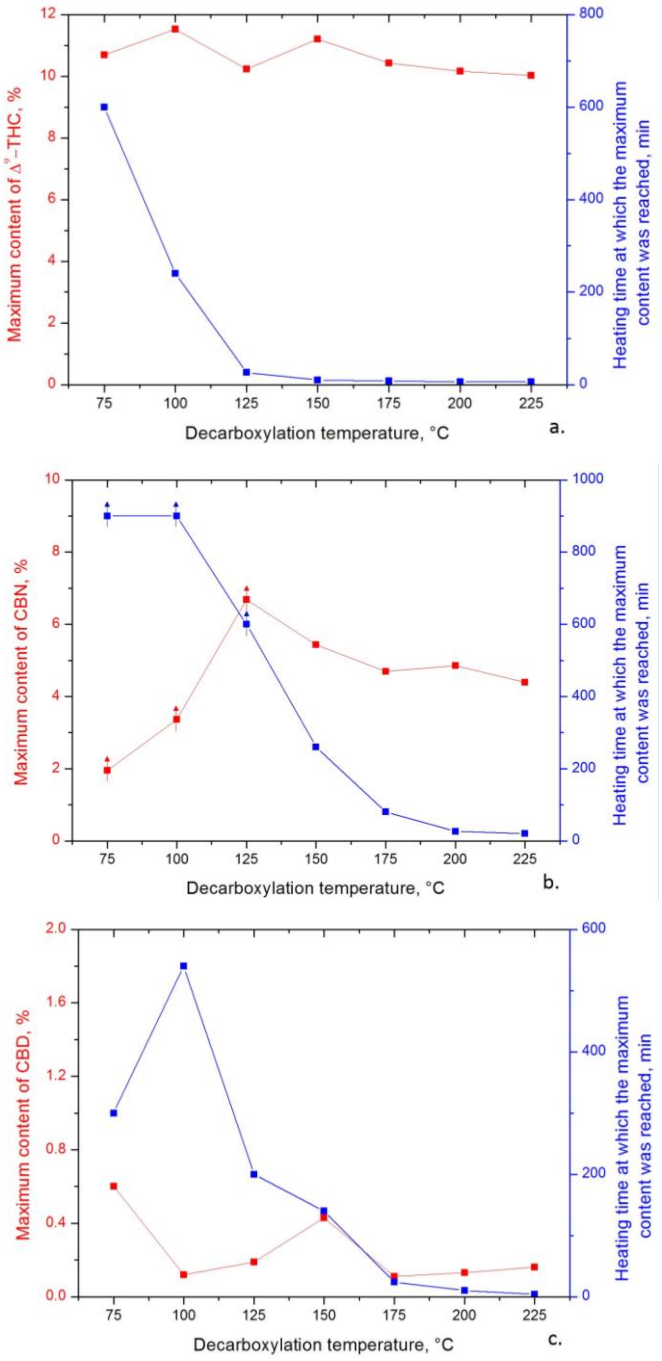


Fig. 5. Variation of the maximum cannabinoids content as well as the heating time at which the maximum content was reached as a function of decarboxylation temperature. The arrows pointing upwards from the graphical representation b. indicate that the maximum content has not been reached, the values represented on the graph corresponding to the maximum heating time at which the experiments were performed.

Regarding the maximum CBN content (only for the temperatures where it was recorded) it generally corresponds to the heating time from which the Δ^9-THC content reaches a minimum value that remains approximately constant regardless of further increase of the heating time (Figs. 4d-g). These results highlight that during the heating time Δ^9-THC is predominantly converted to CBN. Fig. 5b shows a significant decrease in the heating time at which the maximum CBN content was recorded in the temperature range 150 - 225 °C, and also a slight decrease in the maximum CBN content over the same temperature range, which is in accordance with the very low decrease of the maximum Δ^9-THC content recorded over the same temperature range. In the case of CBD, there is no clear tendency regarding the variation of its maximum content according to temperature (Fig. 5c). This could indicate that decarboxylation of CBDA does not only result in the formation of CBD but may also lead to the formation of some unknown side products. The heating temperature at which the maximum content of CBD is reached has a pronounced decreasing trend with the decarboxylation temperature (except for the heating temperature of 100 °C), especially in the temperature range of 150 – 175 °C. Taking into account that the boiling temperature of the CBD is in the range of 160 – 180 °C, it can be considered that at temperatures higher than 150 °C the CBD is mainly removed from the system by evaporation.

4. Conclusions

The experimental results obtained during the investigation of the influence of temperature and heating time on the decarboxylation process of Δ^9-THCA and CBDA from seized cannabis inflorescences showed that the decarboxylation rate increases as the temperature increases. It was also found that the maximum Δ^9-THC content varied marginally with the change of decarboxylation temperature, which indicates a complete conversion of Δ^9-THCA into Δ^9-THC. This is not the case for CBD, where variation of maximum content suggests the formation of some secondary compounds during the decarboxylation process of CBDA. Starting with the decarboxylation temperature of 150 °C, the Δ^9-THC samples content was practically reduced to zero after 10 minutes of heating and corresponds to the heating times at which the maximum CBN content was reached. These results highlight that the Δ^9-THC accumulated in the system during the decarboxylation process is almost completely converted to CBN during heating.

As a general conclusion, strongly supported by the obtained results in this paper, it can be recommended that for a more accurate quantification of the total Δ^9-THC content, the decarboxylation of Δ^9-THCA should be carried out at 150 °C for 10 minutes.

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