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Kinetics of CBD, Δ^9-THC Degradation and Cannabinol Formation in Cannabis Resin at Various Temperature and pH Conditions

Wuttichai Jaidee,[1,2] Ittipon Siridechakorn,[1] Siwames Nessopa,[1] Vanuchawan Wisuitiprot,[1] Nathareen Chaiwangrach,[3] Kornkanok Ingkaninan,[1,3] and Neti Waranuch[1,4,*]

Abstract

Introduction: Cannabidiol (CBD), cannabinol (CBN), and Δ^9-tetrahydrocannabinol (Δ^9-THC) are major cannabinoids in cannabis resin and products. The kinetic of the chemical reaction of resin cannabis is important for product development and storage. A few reports are available in the literature on the rate of CBD and Δ^9-THC degradation, and CBN formation in dried resin and solutions of various pH.

Materials and Methods: Thermal degradation of CBD, Δ^9-THC, and formation of CBN was studied at 50°C, 60°C, 70°C, and 80°C for dried cannabis resin. The effect of pH and temperature on cannabinoids transformation in cannabis solution was also examined at pH 2, 4, 6, 8, 10, and 12 and at 40°C, 50°C, 60°C, and 70°C. High-performance chromatography coupled with diode-array detection (HPLC-DAD) was used for the analysis of CBD, CBN, and Δ^9-THC transformation. The values of activation energies (E_a), shelf-life ($t_{90\%}$ - $t_{110\%}$), and rate constant (k) were calculated for the CBD, Δ^9-THC, and CBN. The effect of temperature and pH on the dried cannabis resin was adequately modeled with the Arrhenius equation.

Results: The results indicated that the chemical kinetics in the thermal degradation of CBD, Δ^9-THC, and formation of CBN were the zero-order, pseudo-zero-order, and first-order reactions, respectively, in cannabis resin. The first-order and pseudo-first-order degradation kinetics were evidenced for CBD and Δ^9-THC, respectively, in cannabis solutions, whereas the zero-order formation kinetic was detected for the CBN. The transformation rate of the CBD, CBN, and Δ^9-THC increased with increasing temperature, especially as temperature increased to 70°C at pH 2.0. The optimum pH for CBD stability was between pH 4 and 6, whereas the optimum pH for Δ^9-THC stability was between pH 4 and 12.

Conclusion: The major cannabinoids (CBD, CBN, and Δ^9-THC) reacted more quickly at high temperature and in an acidic solution. Especially, the minimum transformation of CBD, CBN, and Δ^9-THC was achieved by using on a low temperature, slightly to moderately acidic pH values, and short-time processing. These results may help to improve the storage condition of CBD, CBN, and Δ^9-THC products and in the manufacturing process.

Keywords: cannabis resin; degradation; activation energies; order reaction; Arrhenius equation

Introduction

Cannabis or marijuana

Cannabis sativa L. also knows as Marijuana. It has been categorized as an additive substance and is prohibited in many countries. Cannabis is of interest in medical and health applications such as cosmetics, therapeutic beverages, and other therapeutic agents. The major components are CBD and Δ^9-THC, which present

[1]Cosmetics and Natural Products Research Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand.
[2]Medicinal Plant Innovation Center of Mae Fah Luang University, Mae Fah Luang University, Chiang Rai, Thailand.
[3]Center of Excellence in Cannabis Research, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand.
[4]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok, Thailand.

*Address correspondence to: Neti Waranuch, PhD, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Thapho Sub-district, Muang District, Phitsanulok 65000, Thailand, E-mail: netiw@nu.ac.th

different bioactivities. They, particularly CBD, have inspired these medical and health. Although there is a growing trend in the availability of cannabis applications in the market, the physicochemical properties, including stability behavior, of the major compounds are not yet fully understood.

A report on the degradation rate of CBD and Δ^9-THC and the formation of CBN from cannabis resin (CS) is available. Cannabis contains nearly 500 compounds from 18 chemical classes, but the biological effect of Cannabis derives mainly from naturally occurring phytocannabinoids. One hundred phytocannabinoids have been identified.[1] The most common phytocannabinoids are Δ^9-THC, CBD, cannabigerol, cannabichromene, and CBN.[1–3] CBD, THC, and CBN, have frequently been studied for their degradation kinetics. Their structures are shown in Figure 1. The degradation of CBD through a cyclization reaction resulted in Δ^9-THC, followed by the oxidation of Δ^9-THC to CBN.[4,5] Knowledge regarding the thermal stability of these compounds is required to predict the degradation resulting from exposure to various temperatures and pH levels during unit operations in their clinical use (e.g., metabolism),[6,7] manufacture (e.g., extraction, concentration),[8–14] during incorporation into products (e.g., baking, extrusion processing, pasteurization/sterilization),[1,15] and storage.

Currently, knowledge of the thermal kinetics of CBD, CBN, and Δ^9-THC in CS is limited. Studies of the degradation process of Δ^9-THC found that the double bond of Δ^9-THC may further isomerize to Δ^8-THC as the first-order reaction under a pH value below 4 at 60°C and 70°C.[16] The oxidation of Δ^9-THC to CBN occurred under air exposure.[17] The formation of CBN from Δ^9-THC in ethyl acetate resin solution storage at −18°C was proposed. There was also a report on the acid conversion of CBD to Δ^8-THC and Δ^9-THC when exposed to simulated gastric fluid.[18]

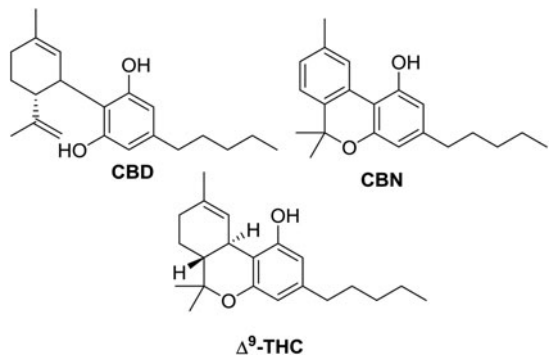


FIG. 1. Structure of the three major cannabinoids.

Although the stability of cannabinoids has been intensively studied, only a few reports are available on the degradation rate of CBD, CBN, and Δ^9-THC in dried resin and pH solution. Therefore, the objectives of this study were to assess the thermal stability of CBD, CBN, and Δ^9-THC in dried CS and solutions of various pH at different temperatures.

Materials and Methods

Chemicals and reagents

These chemicals and reagents were sourced from various suppliers, as follows: high-performance liquid chromatography (HPLC) grade acetonitrile, ethanol, diethyl ether, hexanes, ethyl acetate, and sulfuric acid (RCI Labscan, Bangkok, Thailand), potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH; Ajax Finechem), potassium chloride (KCl; J.T. Baker, Ecatepec de Morelos, Mexico), sodium bicarbonate ($NaHCO_3$), and ammonium formate (NH_4HCO_2; Sigma-Aldrich, St. Louis, MO). Column chromatography was carried out on silica gel 60H (5–40 μm; Merck). Silica gel plates (silica gel 60 F254; Merck) were used for thin layer chromatography. HPLC-grade water was prepared by using Elix essential 5 ultraviolet (UV) + Milli-Q reference A, ultra-purification water systems (Merck Millipore) in series. The mobile phase and the samples were all filtered through a 0.45 μm membrane. The mass spectrometry method parameter and ultra performance liquid chromatography conditions for the dissolution apparatus are listed in Supplementary Table S1.

The standard Δ^9-THC CRM 10 mg/mL solution in ethanol was purchased from Lipomed (purity 98.89% ± 0.036% by HPLC). CBD 1 g (purity 99.9% by HPLC) and CBN 100 mg (purity 99.2% by HPLC) were purchased from THC Pharm GmbH. In addition, dried *C. sativa* was sourced from the Narcotics Suppression Bureau, Royal Thai Police, Bangkok, Thailand.

Plant materials and extraction

C. sativa (258.6 g) was heated at 80°C for 2 h, pulverized, and extracted by sonication at 3 h with 1 L petroleum ether. This was done three times. The filtrated crude extract was further processed by acid-base extraction according to the previous procedure,[19–21] yielding a 2.52 g acid fraction as a brown viscous liquid. The petroleum ether extract was decarboxylated by

heating at 100°C for 3 h for determination of the entire content of major cannabinoids (CBD, CBN, and Δ^9-THC).[22,23] The HPLC chromatogram revealed a loss of cannabidiolic acid, and Δ^9-tetrahydrocannabinolic acid signals in CS (Supplementary Fig. S1). The petroleum ether extract (44.5 g) was fractionated on silica gel 60 (0.063–0.2 mm) and eluted with diethyl ether–hexanes (1:4, *v/v*) to give 10.5 g of a brown viscous liquid of CS. The HPLC chromatogram revealed a very high content of CBD, CBN, and Δ^9-THC in CS (Fig. 2).

Chemical kinetics in various pH solutions

The effect of pH on the chemical stability of the CBD, CBN, and Δ^9-THC in the CS solution was studied at different temperatures, ranging from 50°C to 70°C. Solutions were prepared under various pH conditions (2–12), including pH 2.0 (0.2 M HCl + 0.2 M KCl), pH 4 (0.1 M KH_2PO_4 + 0.2 M HCl), pH 6 (0.1 M KH_2PO_4 + 0.1 M NaOH), pH 8 (0.1 M KH_2PO_4 + 0.1 M NaOH), pH 10 (0.05 M $NaHCO_3$ + 0.1 M NaOH), and pH 12 (0.05 M $NaHCO_3$ + 0.1 M NaOH).

The dried CS (10 mg) was then dissolved in ethanol and mixed with various buffer solutions (3:2, *v/v)* to produce 10 mg/mL of solution.

Each of these solutions was incubated separately at 40–70°C, and then 100 μL of the solutions were transferred at 0, 5, 10, 15, 20, and 25 days to a vial containing 900 μL of ethanol to produce stock solution 1 mg/mL, which was then filtrated, and diluted to 200 μg/mL. This solution was then stored at −20°C until analysis, generally within 24 h. The injection volume was 5 μL for HPLC analysis. The experiment was performed in triplicate.

Chemical kinetics in heat treatment and acidic solution

The CS solutions (1 mg/mL) in 0.2 M KCl, pH 2 were incubated at 40–70°C. Samples of CBN and Δ^9-THC at 40–60°C were then collected at 0, 5, 10, 15, 20, and 25 days. However, CBN and Δ^9-THC that had been maintained at 70°C for 10 days were collected at 2, 4, 6, 8, and 10 days. Sample collection for analysis of CBD was done at 40°C and 50°C for 14 days, which were collected at days 2, 4, 6, 8, 10, 12, and 14; at 60°C this compound was also collected for 4 days at days 0, 1, 1.5, 2, 3, and 4, and at 70°C, for 30 h at times 0, 6, 12, 18, 24, and 30 h. For quantitative analysis of CBD, CBN, and Δ^9-THC in CS solutions, each collected sample was diluted with ethanol to produce a CS solution with a concentration of 1 mg/mL. The quantitative analysis of Δ^9-THC and CBN was done as stock CS solution, which was diluted to 200 and 300 μg/mL for CBD.

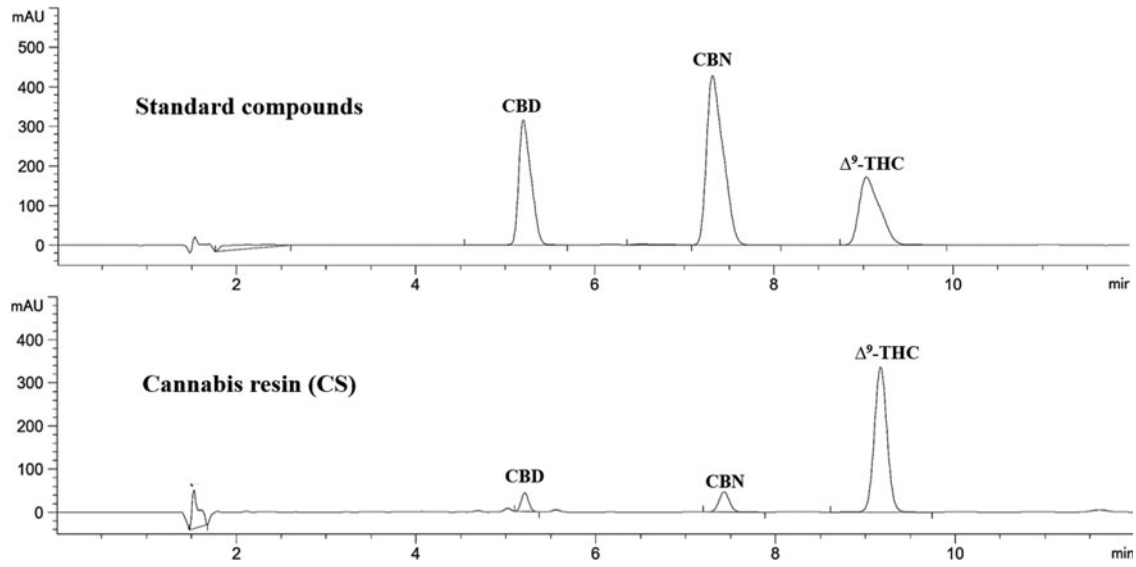


FIG. 2. HPLC chromatograms of the standard mixture of CBD, CBN, and Δ^9-THC (100 μg/mL) and CS (200 μg/mL). CBN, cannabinol; CS, cannabis resin; HPLC, high-performance chromatography.

Chemical kinetics in different temperatures

The CS (50 mg) was weighed into micro-centrifuge tubes. All tubes were protected from light. The sample tubes were kept at different temperatures, ranging from 50°C to 80°C in the oven for 35 days; three sample tubes were collected at days 0, 7, 14, 21, 28, and 35 and then kept at −20°C until they were quantified for the remaining level of test compounds by HPLC. The experiment was performed in triplicate.

Determination of CBD, CBN, and Δ^9-THC in the dried CS was done as follows: 1 mg of dried CS was weighed and diluted with 1 mL of ethanol to produce a stock solution of 1 mg/mL. A stock CS solution was diluted to 200 μg/mL with ethanol and then analyzed with the HPLC system.

Quantitative analysis of compounds CBD, CBN, and Δ^9-THC

The HPLC analysis was performed by using a 1260 Quat Pump VL (Agilent). Chromatographic separations were performed on a reversed-phase column: Kinetex 100°A C18 (4.6 × 150 mm, 2.6 μm; Phenomenex), and they maintained column temperature at 40°C. The UV detector was set at 220 nm. Chromatographic conditions containing the mobile phase were a mixture of solvent 20 mM NH_4HCO_2 pH 3.6 and acetonitrile ratio 25:75, *v/v* with a flow rate of 0.8 mL/min. The samples were filtrated through the 0.45 μm Nylon membrane. The injection amount was 5 μL.

For the standard curve, 1 mg of Δ^9-THC standard was diluted in ethanol to the concentration of 200 μg/mL. This solution was then serially diluted with ethanol for plotting a calibration curve of six concentrations, ranging from 3.1 to 200 μg/mL. The standard curves of CBN and CBD were plotted with five concentrations, ranging from 2.5 to 50 μg/mL. The method was validated to achieve the following parameters: linearity, precision (intraday and interday analysis), accuracy, the limit of detection, and limit of quantification.[24]

Results

HPLC coupled with diode-array detection analysis

The HPLC method for cannabinoid analysis was adapted from the American Herbal Pharmacopoeia.[25] The separation of the major cannabinoids (CBD, CBN, and Δ^9-THC) from CS was achieved within the run time of 12 min. The compounds CBD, CBN, and Δ^9-THC were separated and eluted at t_R 5.21, 7.32, and 9.04 min, respectively. The chromatograms of standards CBD, CBN, and Δ^9-THC and CS are shown in Figure 2.

Kinetics degradation of CBD, Δ^9-THC, and formation of CBN

Determination of the reaction order of the chemical kinetic of CBD, CBN, and Δ^9-THC was done via plotting concentration (C), ln(C), or 1/C against time.[26] Their corresponding correlation efficiencies (r^2) were calculated. The plot of concentration (C), ln(C), or 1/C against the reaction time (day) showed a good correlation ($0.8765 < r^2 < 0.9958$).

The dependence of the rate constant (k) and activation energy (E_a) on temperature was calculated by the Arrhenius equation [Eq. (1)]:

$$ln\ k = -\left(\frac{Ea}{R}\right).\frac{1}{T} + In\ A \tag{1}$$

where k is a specific reaction rate, A is the pre-exponential factor (day^{-1}), E_a is the activation energy (J/mol), T is the absolute temperature (Kelvin), and R is the ideal gas constant (8.31 J/mol K). The plot of the logarithm of the rate constant as a function of $1/T$ would result in a straight line with the negative of the slope equal to E_a/R.

The Arrhenius factors, which are the accelerated breakdown over the tested temperature of each compound, were calculated by using Equation (2):

$$\mathrm{A} = e^x \tag{2}$$

where x is the y-intercept of the semi-logarithmic curve of the Arrhenius plot.

The shelf-life ($t_{90\%}$) for zero- and first-order kinetic was calculated from Equations (3) and (4):

$$t_{90\%} = \frac{0.1 \times [Ao]}{kp}\ \text{(zero-order)} \tag{3}$$

$$t_{90\%} = \frac{-\ln(0.9)}{kp}\ \text{(first-order)} \tag{4}$$

The shelf-life ($t_{110\%}$) for zero- and first-order kinetic was calculated from Equations (5) and (6):

$$t_{110\%} = \frac{0.1 \times [Ao]}{kp}\ \text{(zero-order)} \tag{5}$$

Table 1. Degradation Rate Constants for CBD, Δ^9-THC, and the Formation Rate Constant for Cannabinol in pH 2 Solution, Estimated by the Mean of a First-Order Kinetic Model for CBD, a Zero-Order Kinetic Model for Cannabinol, and a Pseudo-First-Order Model for Δ^9-THC

	Compound					
	CBD		CBN		Δ^9-THC	
Temperature (°C)	k_d (day^{-1})	(r^2)	k_d (μg·mL^{-1}·day^{-1})	(r^2)	k_d (day^{-1})	(r^2)
40	0.0266	0.9526	0.2681	0.9023	0.0183	0.9815
50	0.0453	0.9641	0.6691	0.9503	0.0206	0.9801
60	0.5007	0.9438	0.8090	0.9574	0.0531	0.9313
70	1.6222	0.9902	2.9108	0.9894	0.0928	0.9648

CBN, cannabinol.

$$t_{110\%} = \frac{0.105}{kp} \text{ (first-order)} \quad (6)$$

where A_0 is the initial concentration, and k_p is the rate of the reaction at predicted temperature from the Arrhenius plot curve.

Influence of pH

The changes in CBD, CBN, and Δ^9-THC in different pH buffer (pH 2–12) solutions at 60°C are shown in Supplementary Figures S2–S5. These HPLC chromatograms showed that the kinetic rate led to an increase in acid condition. The HPLC chromatogram of CBN in pH 2, 60°C increased whereas that of Δ^9-THC decreased. Further, the chromatogram of CBD (t_R 5.2 min) disappeared with the appearance signals of impurity I–IV (t_R 3.0, 4.1, 4.9, and 9.2 min) (Supplementary Fig. S4b). All these results indicated a specific acidic catalyzed effect in the degradation of CBD and Δ^9-THC, and the formation of CBN.

Kinetics of CBD, Δ^9-THC degradation, and CBN formation in heat treatment and pH 2 solution

The rate constants of CBD and Δ^9-THC degradation and CBN formation in CS were investigated both at 40–70°C, pH 2, and at 50–80°C, dried resin. Chemical kinetic parameters and profiles for CBD, and CBN shown in Tables 1, 2 and Figures 3, 4. As expected, the apparent kinetic rate constant (k) increased with the increase in temperature. It is clear from Figure 3 that an order reaction of Δ^9-THC agreed with those from the previous studies on kinetics degradation of CS.[3,5] Reporting a pseudo-type reaction model for degradation of Δ^9-THC could be from biochemical and acid-catalyzed cyclization of CBD to Δ^9-THC. The orders of reaction of CBD and CBN were assigned as zero- and first-order reaction, respectively. A strong correlation with the correlation coefficients ($0.9023 < r^2 < 0.9902$) from the plot of concentration (C) or ln (C) against the reaction time (day) was found (Table 1).

The kinetic parameters of CBD, CBN, and Δ^9-THC transformation when heating CS in pH 2 are shown in Table 3. The rate constant obtained from Equation (1) was fitted to an Arrhenius-type equation in each kinetic model studied (Fig. 3) to determine the effect of temperatures on the chemical reaction. The k values showed that the thermal stability of the CBD and Δ^9-THC decreases with increasing temperature.

Influence of temperature

The transformation of CBD, CBN, and Δ^9-THC in dried CS was investigated at 50–80°C for 35 days.

Table 2. Degradation Rate Constants for CBD, Δ^9-THC, and the Formation Rate Constant for Cannabinol in Dried Resin, Estimated by the Mean of a Zero-Order Model for CBD, a First-Order Model for Cannabinol, and a Pseudo-Zero-Order Kinetic Model for Δ^9-THC

	Compound					
	CBD		CBN		Δ^9-THC	
Temperature (°C)	k_d (μg·mL^{-1}·day^{-1})	(r^2)	k_d (day^{-1})	(r^2)	k_d (μg·mL^{-1}·day^{-1})	(r^2)
50	0.1236	0.8765	0.0103	0.9496	1.2444	0.8992
60	0.1268	0.8896	0.0225	0.8782	1.3457	0.9193
70	0.1332	0.9559	0.0356	0.9958	1.4181	0.8887
80	0.1393	0.9223	0.0387	0.9619	1.6313	0.9134

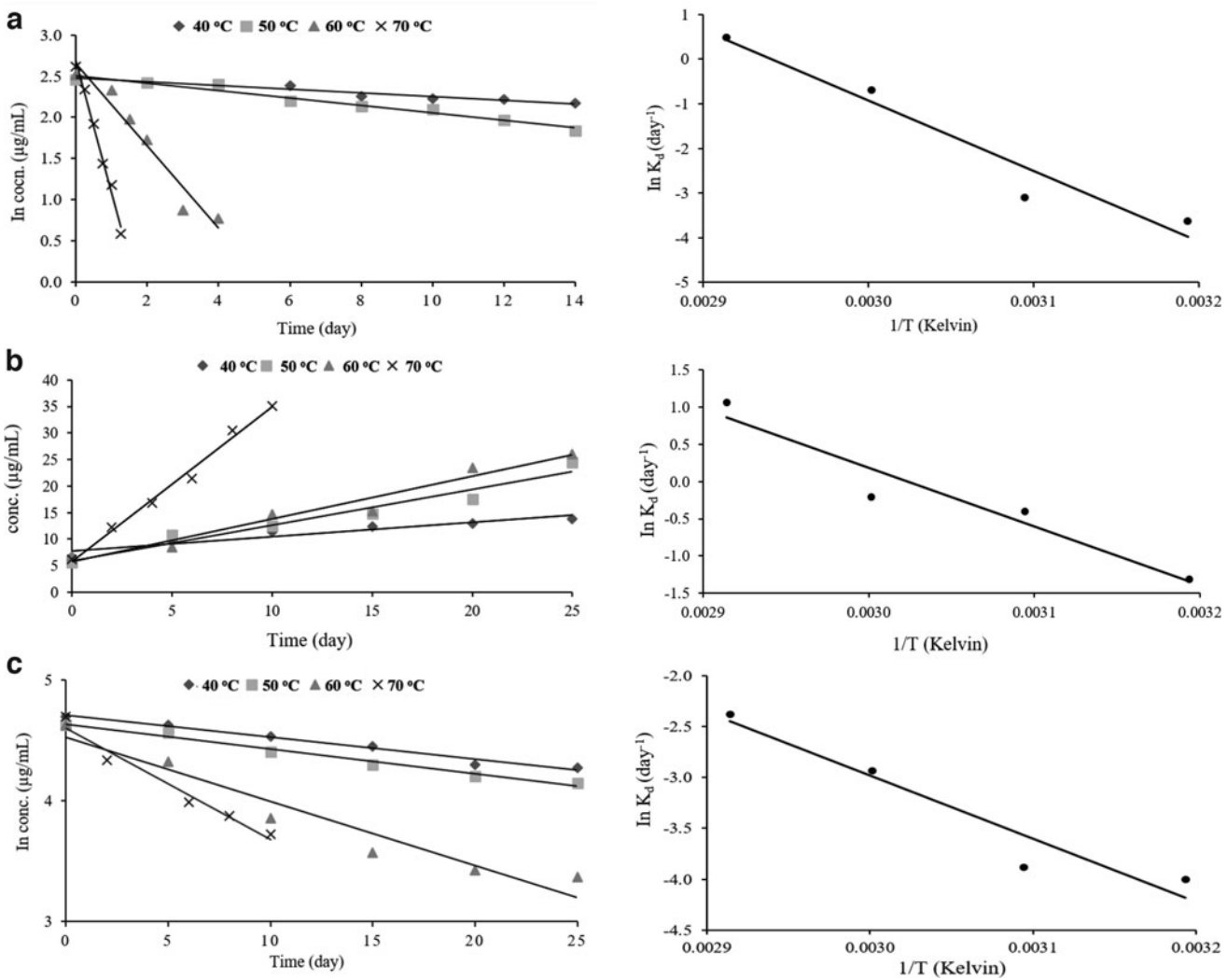


FIG. 3. (**a**) First-order plot of the degradation of CBD, (**b**) zero-order plot of the degradation of CBN, and (**c**) pseudo-first-order plot of the degradation of Δ^9-THC under pH 2 solution over the temperature range 40–70°C and its Arrhenius plots for degradation.

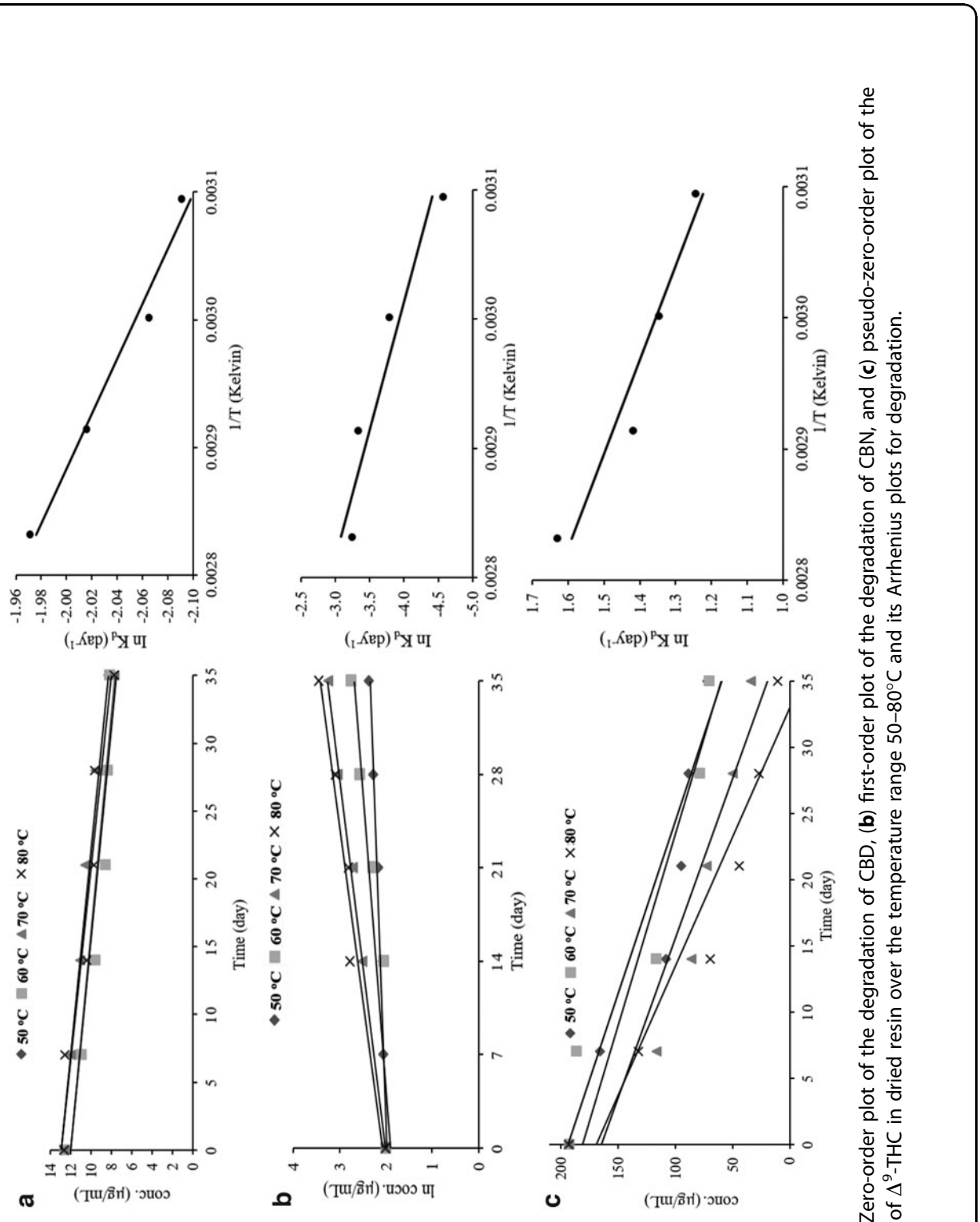


FIG. 4. (**a**) Zero-order plot of the degradation of CBD, (**b**) first-order plot of the degradation of CBN, and (**c**) pseudo-zero-order plot of the degradation of Δ^9-THC in dried resin over the temperature range 50–80°C and its Arrhenius plots for degradation.

Table 3. Predicted Shelf-Life ($t_{90\%}$) of CBD, Δ^9-THC, and Shelf-Life ($t_{110\%}$) of Cannabinol in Dried Cannabis Resin and pH 2 Solution at 25°C Using an Arrhenius Method

	Compound					
	CBD		CBN		Δ^9-THC	
Parameter	Resin[a]	Solution[b]	Resin[b]	Solution[a]	Resin[c]	Solution[d]
E_a (kJ/mol)	3.86	131.21	42.40	65.39	11.63	51.70
A (day^{-1})	0.51	1.43×10^{20}	8.57×10^{4}	2.12×10^{10}	0.25×10^{3}	6.40×10^{6}
k_p at 25°C[e]	0.41	0.0014	0.0032	0.074	2.36	0.0056
$t_{90\%}$ at 25°C (day)	3	71	—	—	8	18
$t_{110\%}$ at 25°C (day)	—	—	32	8	—	—

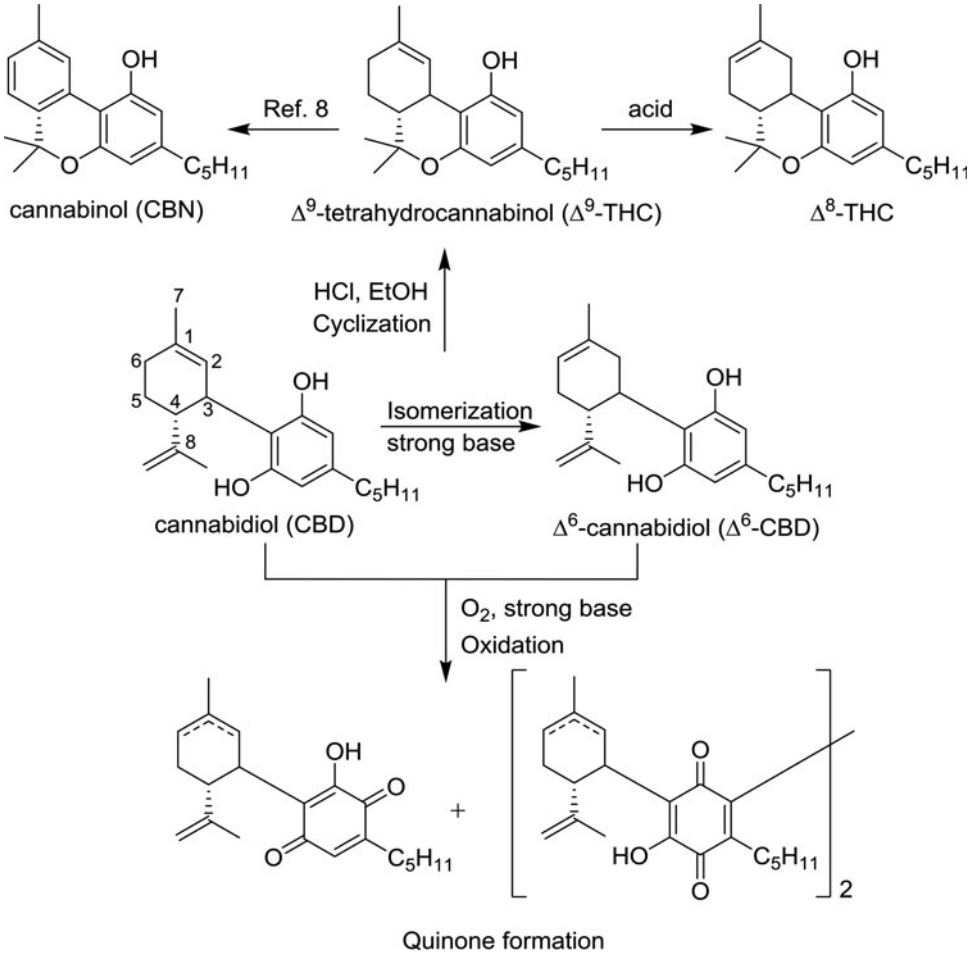
[a]Zero-order degradation.
[b]First-order degradation.
[c]Pseudo-zero-order degradation.
[d]Pseudo-first-order degradation.
[e]Units of rate constants in zero-order degradation = (μg $mL^{-1}\cdot day^{-1}$), pseudo-zero-order degradation = (μg $mL^{-1}\cdot day^{-1}$), pseudo-first-order degradation = (day^{-1}), and first-order degradation = (day^{-1}).
A, pre-exponential factor; E_a, activation energy.

The effect on the transformation of CBD, CBN, and Δ^9-THC from temperatures demonstrated zero-order, first-order, and pseudo-zero-order reaction, respectively. The reaction order of Δ^9-THC in dried CS agreed with the previously reported findings.[5] The effect of temperature on the parameter studied is listed in Tables 2 and 3. These results indicated that the k values increased with increasing temperature (Table 2). The E_a values of CBD, CBN, and Δ^9-THC at 50–80°C were obtained from Equation (1) (Table 3). The shelf-life values at 25°C for CBD, CBN, and Δ^9-THC were 3, 32, and 8 days, respectively.

Discussion

The acid-catalyzed reaction was reported as the cyclization reaction of CBD, leading to Δ^9-THC and iso-THC (Scheme 1).[3,27,28] The Δ^9-THC may further isomerize to Δ^8-THC (Scheme 1).[29] The HPLC chromatogram initially detected a signal of Δ^8-THC within 5 days in CS, and a trace amount of Δ^8-THC showed at t_R 9.2 min (Supplementary Figs. S4b, S6, and S7). The CBN and Δ^9-THC were found to be stable in buffer pH 4–12 for at least 25 days at 60°C, which indicated that CBN and Δ^9-THC are stable in moderately acidic to basic conditions (Supplementary Figs. S2 and S3). The reactions of CBD in basic condition (pH 8–12) presented a violet solution. These results illustrated that the anions of oxidized CBD have a deep violet color because of the Beam reaction, which oxidized to monomeric and dimeric hydroxyquinones (Scheme 1).[3,30] In this study, CBD quinone was found as a type of *p*-benzoquinone that is a class of toxicological intermediates, which can create a variety of hazardous effects *in vivo*, including acute cytotoxicity, immunotoxicity, and carcinogenesis.[31] The liquid chromatography electrospray ionization quadrupole time-of-flight (LC-ESI-QTOF) chromatogram revealed a mass of CBD quinones in CS at t_R 19.6 min (Supplementary Figs. S8 and S9). Therefore, alkaline products of cannabis can be explored as a valuable source of CBD quinone derivatives such as soaps and shampoo. The CBD in a basic condition also led to isomerization at Δ^6-position to produce Δ^6-CBD (Scheme 1).[3,32] These reactions were revealed on HPLC chromatograms of CBD in pH 8–12, which showed a peak of major impurity IV (t_R 5.0 min) instead of CBD (t_R 5.2 min) (Supplementary Fig. S5). The peak of impurity IV was initially detected within 5 days in pH 10 and 12 (results not shown). According to these results, the optimum pH for CBD stability was between pH 4 and 6. However, Δ^9-THC stability was between pH 4 and 12. The stability of Δ^9-THC was also evident from a plot of ln conc. (μg/mL) against times (day) (Supplementary Fig. S2). Therefore, as below pH 4, the degradation rate of both CBD and Δ^9-THC would significantly increase. However, pH values lower than 4 are rarely relevant in food or drugs and their processing.

The CBD was degraded under acidic conditions via the cyclization reaction to produce Δ^9-THC and other psychoactive cannabinoids (Supplementary Fig. S6).[3,18,28] This is the first time that thermal degradation kinetics of CBD in pH 2 solution has been reported. The result correlated with the previous studies reporting a first-order reaction model for the degradation of CBD from a simulated gastric fluid.[20] The calculated E_a from slopes of the line showed that values



SCHEME 1. Chemical reactions of Δ^9-THC and CBD under acidic and basic conditions.

for CBD, CBN, and Δ^9-THC were 131.21, 65.39, and 51.70 kJ/mol, respectively (Table 3). Since high E_a generally indicates, the reaction is less sensitive to temperature changes. Hence, CBD tends to be less sensitive to temperature than CBN and Δ^9-THC. Thus, the CBD in CS solution is more stable to temperature elevation during heating.

Degradation of Δ^9-THC under acid-catalyzed below pH 4 further isomerized to Δ^8-THC.[3,16,27–29,33] The conversion of Δ^9-THC to CBN was widely accepted as the main pathway under acid or heat conditions (Scheme 1).[5,8,22,33] It was confirmed that the peak of Δ^9-THC decreases whereas CBN increases for each 5 day period at pH 2, at 60°C for 25 days (Supplementary Figs. S6 and S7). The study comparing the degradation of Δ^9-THC under temperatures at 40–70°C with the formation of CBN was presented in Figure 3c and b, respectively. The kinetic parameters of CBN formation and Δ^9-THC degradation during heating are shown in Table 3. There was an increasing k value with increasing temperature, showing that the transformation of the CBN and Δ^9-THC increased with increasing temperature (Fig. 3). The E_a values were 65.39 and 51.70 kJ/mol for CBN and Δ^9-THC, respectively. These results indicated that Δ^9-THC is slightly sensitive to temperature than the CBN. The consistent Δ^9-THC degradation in CS solution led to a clear understanding of the kinetics of CBN formation in an acid environment. Since Δ^9-THC degradation demonstrated pseudo-first-order kinetics, the formation of CBN could be conservatively estimated as the inverse of this rate.[18] In this respect, according to Trofin et al., reporting the formation of CBN in cannabis oil during the storage cannot be entirely correlated with

the degradation of Δ^9-THC.[5] These effects resulted from some of the other unknown variables beyond the control, such as the degrading of other cannabinoid compounds and THC-related molecules.[8] This is the first time that the thermal degradation kinetics Δ^9-THC and the formation of CBN from CS in pH 2 solution and heat treatment was reported.

The reaction order of CBN and Δ^9-THC was different. These results confirm no direct correlation between Δ^9-THC degradation and CBN formation.[34] The present results suggest that the different degradation routes of CBD, Δ^9-THC, formation of CBN, and other cannabinoids were more complicated in dried CS.[5] During the experiment, the peak of other impurities disappear on HPLC chromatograms, however, we observed the peak of Δ^9-THC decrease whereas CBN increased for each 10°C rise in temperatures (Supplementary Fig. S10), which were, in part, consistent with those obtained from Δ^9-THC. The calculated E_a of dried CS from Equation (1) was less than CS solution and indicated that the substrate in dried CS with lower E_a values tends to be more sensitive to temperature. The E_a of Δ^9-THC in dried CS was more than CBD, suggesting that Δ^9-THC are more stable to high temperature (Fig. 4). The results of this research demonstrate Δ^9-THC under ambient environmental pH and temperature conditions.

Conclusion

The chemical reactions of CBD, CBN, and Δ^9-THC from the CS with different pH levels (pH 2–12), acidic solutions (pH 2 for 40–70°C), and temperatures (50–80°C) were investigated. The optimum pH for CBD stability was between pH 4 and 6, whereas Δ^9-THC stability was achieved between pH 4 and 12. The conversion of Δ^9-THC to CBN was prominent in strongly acidic and high-temperature conditions. The chemical kinetics of CBD, CBN, and Δ^9-THC were pseudo-zero-order, pseudo-first-order, zero-order, and first-order reactions. The transformation of CBD, CBN, and Δ^9-THC depended on temperature and pH, reacting more quickly at high temperatures and acidic conditions, especially at 70°C and pH 2. Thus, the minimum transformation of CBD, CBN, and Δ^9-THC was achieved by using a lower temperature, slightly to moderately acidic pH values, and short-time pressing. These results will help to improve the storage conditions of CBD, CBN, and Δ^9-THC products and in the manufacturing process, especially foods and alkaline conditions of cosmetic products.

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Author Disclosure Statement

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Supplementary Material

Supplementary Table S1
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S5
Supplementary Figure S6
Supplementary Figure S7
Supplementary Figure S8
Supplementary Figure S9
Supplementary Figure S10

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Abbreviations Used

A = pre-exponential factor
CBD = cannabidiol
CBN = cannabinol
CS = resin cannabis
E_a = activation energy
HPLC = high-performance chromatography
k = rate constant
KCl = potassium chloride
KH_2PO_4 = potassium dihydrogen phosphate
$NaHCO_3$ = sodium bicarbonate
NaOH = sodium hydroxide
NH_4HCO_2 = ammonium formate
THC = tetrahydrocannabinol
UV = ultraviolet