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Stability of cannabinoids in dried cannabis: a kinetic study

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Abstract. This study was undertaken to explore the effect of temperature on the degradation of cannabinoids in dried cannabis flower. A total of 14 cannabinoids were monitored using liquid chromatography - tandem mass spectrometry in temperature environments from −20 °C to +40 °C lasting up to one year. We find that a network of first-order degradation reactions is well-suited to model the observed changes for all cannabinoids. While most studies focus on high-temperature effects on the cannabinoids, this study provides high-precision quantitative assessment of room-temperature kinetics with applications to shelf-life predictions and age estimate of cannabis products.

Keywords: Cannabis; stability of cannabinoids; Bayesian kinetic modeling

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1. Introduction

A number of countries have established medicinal cannabis programs and a handful have also legalized cannabis for adult recreational purposes. Regulated cannabis markets typically have stringent testing requirements to ensure the safety of consumers. In addition to measuring cannabinoid and terpene levels, which define the desired properties of the product, cannabis is generally tested for a wide variety of other chemicals including pesticides, mycotoxins, mold, bacteria, and heavy metals.

Determination of cannabinoid levels for regulatory purposes, also known as measuring 'potency', calls for measurements of the major cannabinoids such as the Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and their carboxylic acid precursors (Δ^9-THCA and CBDA). Over 100 other minor cannabinoids are known and some of them are part of mandatory testing in jurisdictions such as New Hampshire or California. Mostly, however, the minor cannabinoid profile is used for branding or exploratory research purposes.

A feature common to all cannabinoids is that they undergo decomposition, most notably the decarboxylation. Although this natural degradation of cannabinoids is well-known, it is generally viewed as inconsequential because the degradation product is an active ingredient. Some regulations take this into account and require reporting 'total THC' which is the sum of Δ^9-THC and Δ^9-THCA defined as $m(\mathrm{THC}) + 0.877m(\mathrm{THCA})$ [1]. However, the decomposition of cannabinoids does not stop with decarboxylation; there is ample evidence [2,3] (supported by the results of this study) showing that THC levels too can decrease over time thus further adding to the overall loss of THC.

Many studies have been devoted to better understand the degradation of cannabinoids[2,3]. This helps to provide evidence-based considerations for the shelf-life of cannabis and is an important part of metrology surrounding cannabis testing in general. A variety of cannabinoid ratio markers are employed to perform chemical characterization of cannabis [4]. As an example, CBN to THC ratio is used as a marker of age [5] or the CBDA to CBD ratio which is used to evaluate storage conditions [6].

Despite the interest in cannabinoids, many studies devoted to measurements of cannabinoids do not distinguish between the carboxylic cannabinoids and their decarboxylated analogues. Unlike liquid chromatography, gas chromatography is known to induce decarboxylation in the injection port [7]. Such conversion is often incomplete and therefore comparing the cannabinoid measurements by gas and liquid chromatography might not be reliable. While others employ a decarboxylation step prior to analysis, such methods can provide biased results.

Table 1. Cannabinoids studied in this work along with their approximate certified mass fraction values in the dried cannabis NRC certified reference material.

Cannabinoid, E	Symbol	$w(\mathrm{E})$, g/kg
Δ^9-tetrahydrocannabinol	THC	60
Δ^9-tetrahydrocannabinolic acid	THCA	125
cannabidiol	CBD	9.1
cannabidiolic acid	CBDA	24
cannabigerol	CBG	2.1
cannabigerolic acid	CBGA	4.3
cannabinol	CBN	2.9
cannabinolic acid	CBNA	2.0
cannabichromene	CBC	1.1
cannabichromenic acid	CBCA	2.0
tetrahydrocannabivarin	THCV	0.3
tetrahydrocannabivarinic acid	THCVA	0.5
cannabidivarin	CBDV	0.05
cannabidivarinic acid	CBDVA	0.14

This study provides high-precision measurements of cannabinoids along with a long-term stability study of these compounds in dried cannabis material using isotopic internal standards. This work was made possible with the development of a cannabis reference material, which had sufficient homogeneity to allow for detection of small changes in cannabinoid levels across samples at different storage conditions.

2. Experimental

2.1. Cannabis material

The cannabis material used in this study was produced by blending two strains of *Cannabis sativa*. One strain contained predominantly THCA-THC and the other CBDA-CBD. Both strains were blended to achieve typical cannabinoid levels (shown in Table 1) using a two-stage blending process using food blenders while ensuring that the material remained at or below ambient temperature. In both stages, blending was followed by passing the material through a 355 μm sieve. The sieved cannabis material was homogenized by hand-mixing and shaking.

2.2. Determination of cannabiniods by LC-MS/MS

The cannabinoid method employed has been described elsewhere [8], and was adapted to yield higher precision and accuracy for this work by using narrower calibration ranges. Briefly, the cannabinoids were extracted from 200 mg cannabis sample using liquid-solid extraction procedure with methanol-water mixture (80:20 volume ratio). The cannabis samples

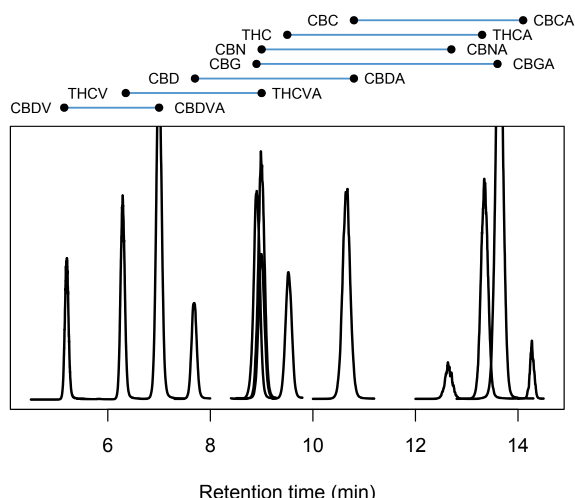


Figure 1. A representative LC-MS/MS chromatogram of a 14 cannabinoid mixture each at 1.0 μg/mL. The image shown is a composite of nine separate chromatograms each corresponding to a unique MS/MS transition.

were extracted sequentially four times (10 mL each time) and supernatants combined to ensure complete extraction. The combined supernatants were vortex-mixed and diluted 100-fold in methanol (we refer to this sample as the diluted cannabis extract).

The LC-MS/MS system consisted of a HPLC system (Infinity, Agilent) coupled to a triple quadrupole mass spectrometer (TSQ Quantiva; Thermo Scientific). Chromatographic separation was carried out on C18-Amide bonded phase column (100 mm × 2.1 mm i.d. with 3 μm particle size) maintained at 40 °C temperature and mobile phases consisting of water/formic acid and acetonitrile/formic acid both mixed in 1000:1 volume ratio. The MS/MS detection of cannabinoids was performed via electrospray ionization in positive ion mode using molecular ion to product ion transitions as outlined elsewhere [8]. The total chromatographic run time was 21 min. External calibration standard solutions of cannabinoids were prepared gravimetrically in methanol in four separate groups to decrease possible cannabinoid inter-conversion bias. Standards were prepared at three levels: at half, double, and same levels of cannabinoids expected in the diluted cannabis extract. Internal isotopic standards were added to aliquots of all cannabis standards and diluted cannabis extracts prior to triplicate injection on the LC-MS/MS system. Unweighted ordinary linear regression was used for calibration with peak area ratio of cannabinoid and internal standard as the response variable. In all cases, calibration curves had $R^2 \geq 0.99$ resulting in a typical combined relative measurement uncertainty of 2 % for all cannabinoids.

2.3. Stability study design

An accelerated isochronous stability study [9] was performed with two units of the CRM stored as each of the temperature levels (−20 °C, +4 °C, +20 °C, +32 °C, +37 °C, and +40 °C) for a duration of up to 52 weeks. Two additional samples were stored at −80 °C serving as the control environment. At each time-point, the CRM units were removed from storage and allowed to equilibrate to room temperature for 1 h. Each CRM unit was then mixed by hand and two 200 mg sample aliquots were transferred into clean 15 mL polypropylene tube with the exact sample masses recorded. The CRM units were then closed and returned to the appropriate storage temperature whereas the weighed cannabis aliquots were stored at −80 °C until the completion of the 50 week term when they were analyzed on the same day. Additional study was done afterwards to provide data for +30 °C and +40 °C temperatures. For this, a pooled sample from three units of the CRM were sub-sampled for +30 °C (1.0 g), +40 °C (1.0 g), and −80 °C control level (0.5 g). At appropriate time points, two 100 mg sample aliquots were transferred into clean polypropylene tubes and stored at −80 °C until analysis.

The overall dataset consists of 924 observations involving seven cannabinoids in their carboxylic and neutral forms (Table 1). Considering that the mass of dried cannabis material is not conserved during the storage of cannabis due to the release of CO_2 as a result of decarboxylation of its components, all samples were weighed after being held at the various temperature environments and all measurement results are expressed as mass fractions of cannabinoids relative to the mass of cannabis at the time of analysis.

2.4. Sample homogeneity

Cannabis plants are extremely inhomogeneous with respect to their cannabinoid content and dried materials require thorough blending [10]. To evaluate the homogeneity of the cannabis sample with respect to its cannabinoid content, we conducted measurements of 14 cannabinoids from 10 CRM units. Uncertainty due to homogeneity, the combination of the between- and within-unit random effects, averaged to 2 % ranging from 1 % (CBGA) to 4 % (CBN). Thus, the cannabis CRM was shown to be homogeneous at the level of measurement uncertainty.

3. Results

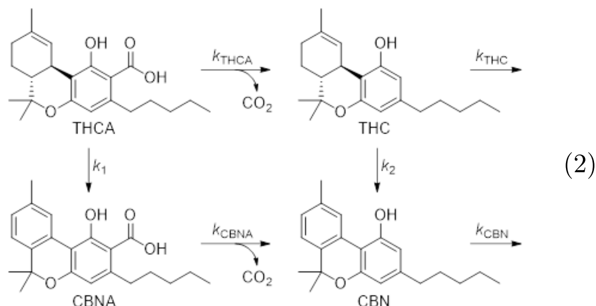
3.1. Kinetic model of cannabinoid stability

Cannabinoids are aromatic carboxylic acids which are capable of decarboxylation in dried cannabis [11]. The conversion of THCA into THC over time is

an example of such a process. Thus, we consider the following idealized consecutive pseudo first-order reaction system to model the changes of cannabinoids:

$$\mathrm{A} \xrightarrow{k_\mathrm{A}} \mathrm{N} \xrightarrow{k_\mathrm{N}} \ldots \quad (1)$$

Here, A refers to the cannabinoid in carboxylic form, N refers to its decarboxylated (neutral) analogue, and Y is the unknown degradation product of N. As an example, tetrahydrocannabinolic acid (A = THCA) converts into tetrahydrocannabinol (N = THC). In addition, we consider the formation of cannabinol (CBN) and cannabinolic acid (CBNA):



(2)

Additionally, the conversion of CBD to THC has been described in the presence of acids at high temperatures [12] but this reaction is unlikely to occur to any significant extent at normal temperatures. In addition, we have not observed the formation of Δ^8-THC in this study. Although other inter-conversions of cannabinoids can occur in plants, such as CBGA → CBDA or CBGA → THCA [13], these enzymatic processes are unlikely to occur in dried plant material. In addition, due to trace amounts of cannabicyclol (CBL) we also disregard the conversion of CBC to CBL [14].

The measurement models are based on the above chemical reactions and can be expressed in a system of ordinary differential equations assuming first-order kinetics. In the simplest two-component model, the decarboxylation of cannabinoids, shown in Eq. 1, can be described using the following system of differential equations:

$$\begin{aligned} \frac{dc_\mathrm{A}}{dt} &= -k_\mathrm{A} c_\mathrm{A} \\ \frac{dc_\mathrm{N}}{dt} &= +k_\mathrm{A} c_\mathrm{A} - k_\mathrm{N} c_\mathrm{N} \end{aligned} \quad (3)$$

The Arrhenius equation has been widely used to model the temperature effect on the rate of chemical reactions:

$$\ln k_\mathrm{A}(T) = a_\mathrm{A} + b_\mathrm{A}/T \quad (4)$$

Thus, for example, the stability of CBDA and CBD in cannabis with respect to time and temperature can be described using a kinetic model with four parameters a_CBDA, b_CBDA, a_CBD, and b_CBD. Our kinetic model was developed with the aim to study cannabinoid changes under storage conditions in dark conditions and does not take into account other physical influences such as the humidity, bacterial activity, pH changes, or light which might play significant role in other applications.

3.2. Model fitting

Given the complexity of the measurement model, we have adopted a Bayesian multi-curve fitting using Markov chain Monte Carlo method [15]. The reaction rate constants are parameters of differential equations which require integration in order to obtain the concentration of substances at any given time and temperature. This can be achieved using either numerical integration methods or Eq. 3 can be integrated analytically to obtain an explicit measurement equation. For simple two-compound systems, such as CBDA-CBD, the analytical solution of the differential equations (Eq. 3) is as follows:

$$\begin{aligned} c_\mathrm{A}(T,t) &= f_\mathrm{A}[k_\mathrm{A}(T),t] \\ c_\mathrm{N}(T,t) &= f_\mathrm{N}[k_\mathrm{N}(T),t] \end{aligned} \quad (5)$$

Closed-form expressions are available for the system of consecutive first-order chemical reactions:

$$\begin{aligned} c_\mathrm{A} &= f_\mathrm{A}[k_\mathrm{A}(T),t] = c_{\mathrm{A},0} e^{-k_\mathrm{A} t} \\ c_\mathrm{N} &= f_\mathrm{N}[k_\mathrm{N}(T),t] = \\ &= c_{\mathrm{A},0}(e^{-k_\mathrm{A} t} - e^{-k_\mathrm{N} t}) \frac{k_\mathrm{A}}{k_\mathrm{N} - k_\mathrm{A}} + c_{\mathrm{N},0} e^{-k_\mathrm{N} t} \end{aligned} \quad (6)$$

The Bayesian model fitting expresses the observed cannabinoid concentrations with observational equations which explain them probabilistically in terms of model parameters, their uncertainties, as well as the uncertainty due to measurement of the cannabinoids, $u(c_\mathrm{A})$ and $u(c_\mathrm{N})$.

Overall, our approach seeks not to find the best fits to each and every kinetic profile. Rather, the fitting involves finding best values of the Arrhenius parameters that best fit the entire data over all temperature environments.

Fitting complex nonlinear kinetic models is often faced with difficulties that necessitate parameter transformation or scaling [16] whereas Bayesian methods can naturally handle parameter estimates in nonlinear models [17]. Additionally, Bayesian framework provides uncertainty distributions for all model parameters and any derived quantities. As an example, one can readily evaluate and make predictions for the total THC content (sum of THCA and THC), ratio of THCA and THC, or the ratio of THC and CBD. Thus, any of these derived quantities

can be modeled as a function of temperature and time while seamlessly accounting for asymmetric parameter uncertainties, constraints imposed by the model, or the correlations between parameters. Moreover, additional uncertainties can be introduced into the model as necessary. These include, for example, uncertainty arising from the temperature control or from the homogeneity of the material itself.

Given the complexity of the analytical expressions, we employed numerical methods to solve (integrate) the system of differential equations and to find the best estimates of all parameters that fit the data. We employed Bayesian methods for this purpose. Typically, the implementation of Bayesian data analysis requires specialized software. The three most common open-source options for Bayesian inference are BUGS, JAGS, and STAN all of which involve Markov chain Monte Carlo methods [15]. We chose STAN largely due to its built-in ability to handle differential equations [18]. The calculations were performed in R using package "rstan" to interface with STAN [19]. All computational codes discussed in this manuscript are provided in the supplementary information.

Bayesian analysis requires prior probability distributions for all parameters. We have adopted diffuse priors to express vague information about all parameters with common-sense boundaries (all mass fractions must be positive and all reaction rates must increase with temperature). It takes approx. 30 min to complete standard fitting routine for CBDA-CBD on a 2 GHz laptop and default settings. Parallel computing was adopted using multi-core processors which reduced the total running time in half on a 4-core processor. The fitted kinetic models for all cannabinoids were incorporated into a interactive web application io order to facilitate the exploration of the results.

The initial values for the model parameters were obtained by pre-fitting the kinetic models to data using R package "mkin" [20] whereas additional analytical calculations developed in this work were aided by *Mathematica* v.10.0.

4. Discussion

We performed the stability study of seven cannabinoids in a dried cannabis material stored in dark environments at temperatures ranging from −20 °C to +40 °C for up to one year. Samples stored at −80 °C were treated as reference and all results are summarized in the Supplementary Information. We performed Bayesian model fitting and Fig. 2 shows the changes in the mass fraction of THC and CBD at several temperatures. Fig. 3 shows the relationship of the decarboxylation rate constants as a function of temperature (the Arrhenius plot). The analysis of these results shows that decarboxylation rate constants of all seven cannabinoids analyzed are indistinguishable from one another in many cases. Thus, given the complexity of the THC-CBN kinetic network, one can impose this observation (i.e, $k_{\mathrm{A,THCA}} = k_{\mathrm{A,CBNA}}$) to facilitate the model fitting for THC.

The observed changes in the mass fraction of cannabinoids align well with the simple first-order kinetic model. We also note the clear opposing trends in the decay of cannabinoid acid forms and the rise of the neutral analogues. These two processes can, and indeed do, cancel each other out: when stored at +40 °C temperature for two weeks (Fig. 2), the total THC equivalent remains nearly unaffected despite the significant loss of THCA. This interplay further demonstrates that the term 'THC' remains too ambiguous without explicit mention of the acid precursor.

4.1. The shelf-life of cannabis

The half-life of the decarboxylation reaction is the time in which half of the carboxylic acid (A) is decarboxylated into the neutral analogue (N). Given first-order decay of A, the time at which the levels of A are at fraction h such as 85 %) of its initial value is given by the following equation:

$$t_{\mathrm{S}} = -\ln h / k_{\mathrm{A}} \tag{7}$$

Generally speaking, we find that the 85 % shelf-life ($h = 0.85$) doubles with each 5 °C reduction in the storage temperature.

Of high practical interest is also the minimum duration, t_{S}, at which the total amount of a cannabinoid has decayed to a certain amount, such as 15 %, below its original value. For THC, as an example, such shelf-life (shown in Fig. 4) is obtained by solving the following expression for t_{S}:

$$\frac{n_{\mathrm{THCA}}(t_{\mathrm{S}}) + n_{\mathrm{THC}}(t_{\mathrm{S}})}{n_{\mathrm{THCA}}(t=0) + n_{\mathrm{THC}}(t=0)} = 0.85 \tag{8}$$

In the early cannabis stability studies, Lerner showed that the THC content of cannabis decreases at the rate of 3−5 % per month at room temperature [21]. Similarly, Zamengo et al showed recently that the average THC degradation in the first 100 days is 12 % at 22 °C (or 3−4 % per month) [22]. Our study is in general agreement with these estimates and puts the average monthly THCA+THC degradation rate at 2 % in 20 °C temperature. While room temperature is indeed unsuitable for storing cannabis standards we have observed that even +4 °C refrigeration falls short to maintain a reasonable long-term stability (Fig. 4).

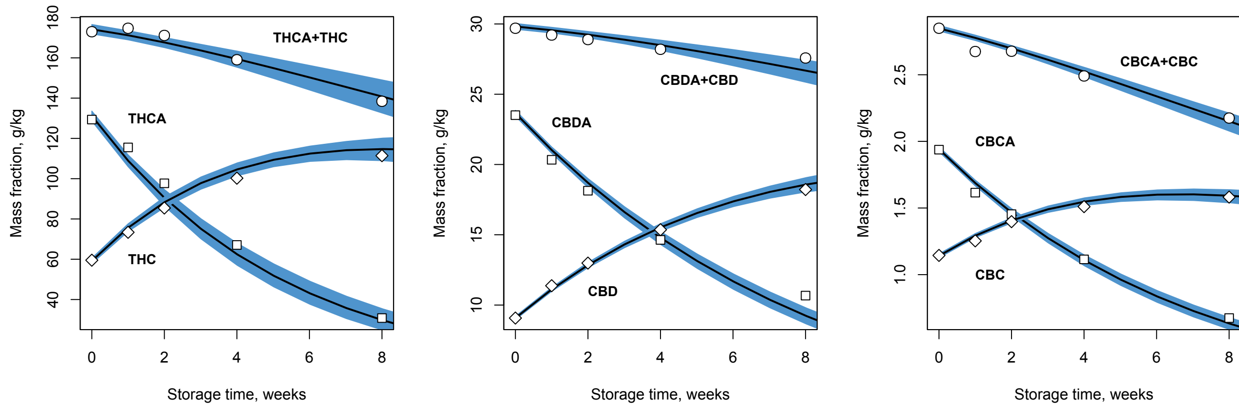


Figure 2. Degradation profiles of THCA-THC, CBDA-CBD, and CBCA-CBC in dried cannabis reference sample stored at +40 °C temperature. The total cannabinoid equivalent (upper lines) are defined, for example, as $m_{\mathrm{THC+THCA}} = m_{\mathrm{THC}} + m_{\mathrm{THCA}}(M_{\mathrm{THC}}/M_{\mathrm{THCA}})$. Black curves represent the best fit of the kinetic model and the surrounding blue segments are the 95 % credible intervals.

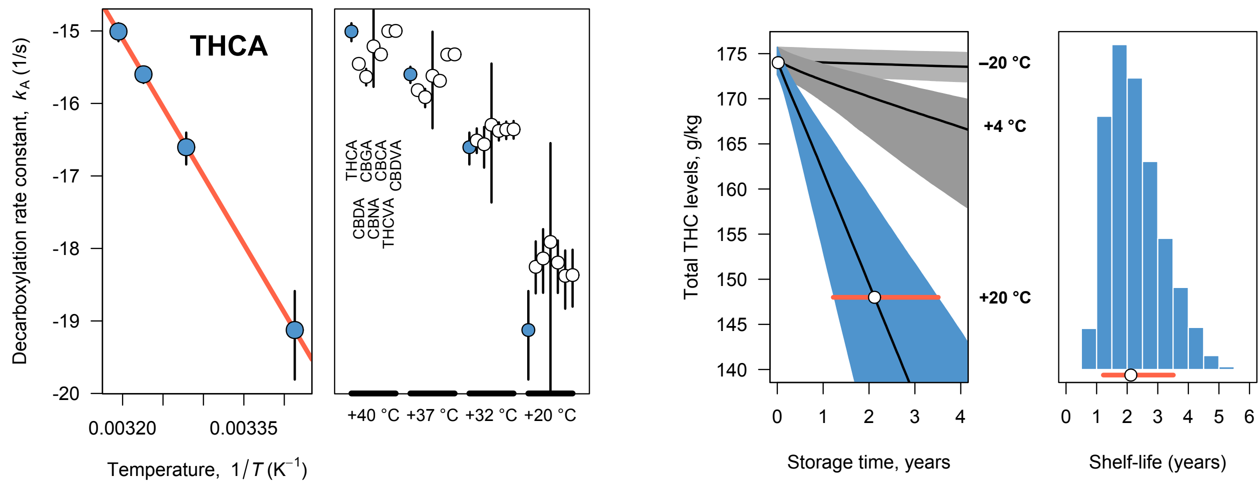


Figure 3. Decarboxylation rate constants of THCA (left) and other cannabinoids (right) in dried cannabis reference material. Vertical lines represent the 95 % credible intervals.

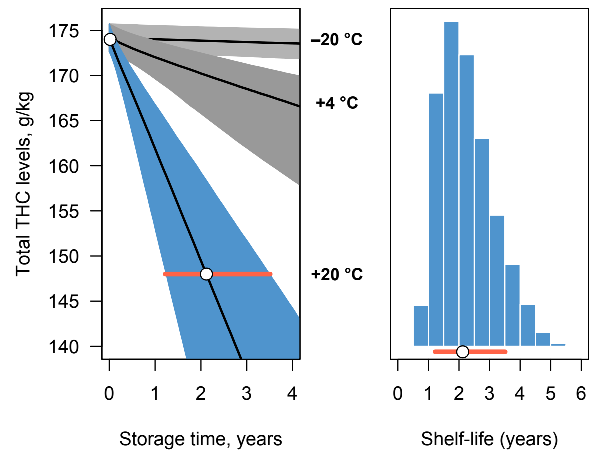


Figure 4. Changes in the total THC equivalent content in dried cannabis reference material stored at +20 °C, +4 °C, and −20 °C (left) and the predicted shelf-life (85 %) of the total THC equivalent at +20 °C (right). Segments around the best fit model lines represent the 90 % credible intervals.

4.2. The age of cannabis

Whereas the shelf-life calculation aims to predict the subsequent changes after the sample analysis, a useful application of the kinetic modeling is to estimate the age of cannabis. We start with the assumption that cannabinoids are present *in vivo* only in their acidic forms (such as THCA, CBDA, or CBCA) which are subsequently decarboxylated upon drying [13, 14, 23]. In this vein, the the ratio of acid-to-neutral form of a cannabinoid provides an internal clock to estimate the age of cannabis whereby the model age of a cannabis sample is defined as the duration (at a given constant temperature) required to reach the observed amount ratio of the neutral and acid forms of a cannabinoid, $R_{\mathrm{N:A}}$. The system of first-order linear differential equations represented in Eq. 1 and 2 can be solved analytically to obtain a closed-form expression for the cannabinoid levels as shown in Eq. 6. Solving the latter for the cannabis model age, t_0, gives the following expression:

$$t_0 = \frac{1}{\Delta k} \ln\left(1 + R_{\mathrm{N:A}} \frac{\Delta k}{k_{\mathrm{A}}}\right) \approx \frac{R_{\mathrm{N:A}}}{k_{\mathrm{A}}} \qquad (9)$$

where $\Delta k = k_{\mathrm{A}} - k_{\mathrm{N}} + k_1 - k_2$. (Note that $k_1 = k_2 = 0$ for all cannabinoids except the THCA/THC and Eq. 9 is not applicable for CBNA/CBN although applicable expressions can be derived.) Measurements of six acidic/neutral cannabinoid ratios in the cannabis CRM provides consistent model age estimates which

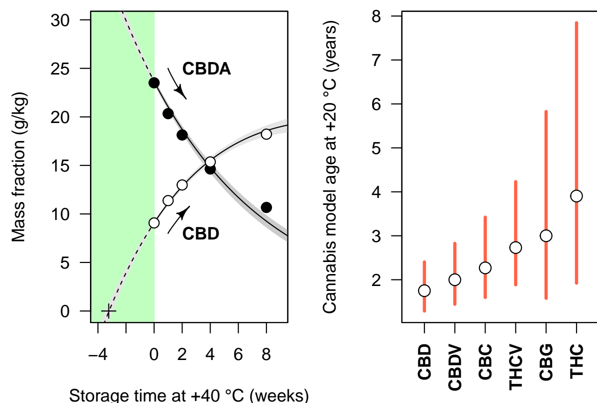


Figure 5. The principle of cannabis model age calculation exemplified with CBD measurements of the cannabis CRM stored at +40 °C temperature (left). The model age, $t_0(40\ °C)$ in this example, is obtained by extrapolating the CBD curve to $w(\mathrm{CBD}) = 0$. Smooth lines represent the best fit of the kinetic model to the data and the surrounding segments are the 95 % credible intervals. Figure to the right shows the +20 °C model age estimates of our cannabis CRM as calculated from the measurements of acid/neutral cannabinoid ratios of six cannabinoids.

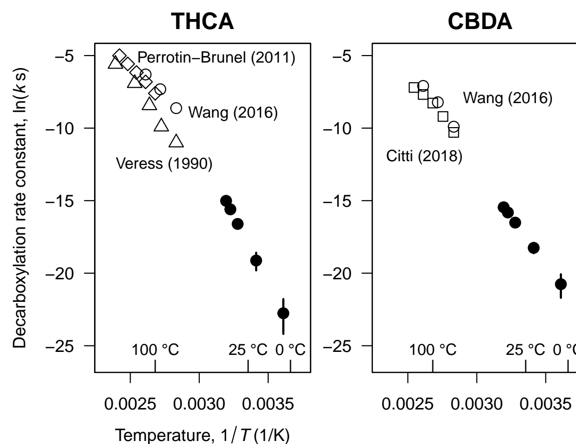


Figure 6. Compilation of THCA and CBDA decarboxylation rate constants reported in the literature [6, 11, 24, 25]. Results from this work are shown with black circles with 95 % uncertainty bars.

are shown in Fig. 5. The contextual knowledge about the provenance of the CRM puts its age estimates to approx. 2 years which is in agreement with our model age estimates.

Given that THCA and THC are known to oxidize to CBNA and CBN over time, the CBN:THC ratio has been used to infer the age of cannabis samples [5]. Most notably, Ross and Elsohly have shown that a simple linear relationship is observed between the cannabis storage duration (t) and the CBN:THC ratio ($R_{\mathrm{CBN:THC}}$), $t \approx 30 \cdot R_{\mathrm{CBN:THC}}$ years, at room temperature [5]. Although this work is ambiguous on the exact meaning of 'THC' and 'CBN', if we interpret them as total equivalents, our results align very well with the classical 'UN formula'.

Indeed, the system of differential equations represented in Eq. 2 can be solved to obtain expressions for the cannabis model age from the ratios involving CBN/CBNA and THCA/THC. For example, $t_0 = \ln(1 + R_{\mathrm{CBNA:THCA}})/k_1$. Note that the measured cannabinoid ratio requires attention to its explicit definition, whether it is CBNA:THCA, CBN:THC, or something else. Similar as with the ratio CBDA:CBD (Fig. 5), extrapolation to zero amount ratio or in our cannabis sample, suggests significantly lower model ages compared to the estimates from other cannabinoids: 9 months from (CBNA+CBN):(THCA+THC) and 17 months from CBDA:THCA. The reasons for this discrepancy are unclear and it remains to be seen which of these age estimates are more robust when applied to wide variety of cannabis samples.

4.3. Comparison with other studies

Many studies have been devoted to decarboxylation of cannabinoids in dried cannabis [6, 11, 24, 25] focusing on high-temperature (ca. 100 °C) and short duration (minutes) domain. In contrast, our study focuses on lower temperatures (ca. 20 °C) and longer time periods (weeks) which is possible, in part, due to the availability of homogeneous cannabis reference material combined with high-precision LC-MS/MS measurements.

While there is a reasonable alignment of the decarboxylation rate constants from these studies, we note that the interpretation of cannabinoid measurements is challenging due to the fact that many studies do not distinguish between the cannabinoids in their acid and neutral form. In fact, some legislative documents still suffer from a similar lack of specificity which has led to many concerns [26].

In closing, an interesting chemical curiosity is the observation that CBN remains the prevalent cannabinoid in century old dried cannabis samples [3], which is consistent with the projection of our kinetic model.

5. Conclusions

Our study explores the behaviour of dry cannabis at storage conditions near room temperature. To this effect, we have conducted high-precision measurements of seven cannabinoids and shown that a simple pseudo first-order kinetic model explains the changes of both

major and minor cannabinoids under the variety of storage conditions ranging from −20 °C to +40 °C. The decarboxylation rate constants agree well with recent other studies conducted at higher temperatures which suggests the wider applicability of our kinetic model to a wide variety of cannabis samples with applications to shelf-life modeling or cannabis age calculations. These results from our study could help inform cannabis regulators in setting degradation thresholds or shelf-lives, and could lay the framework for standardization of stability testing in the cannabis industry [27] [28]. Future studies will aim to evaluate how closely the kinetic parameters of cannabinoid stability in dried cannabis samples aligns with those in hemp or cannabis oils.

Associated Content

The Supporting Information is available free of charge. In addition, the fitted kinetic model is currently available in the form of interactive web application at metrology.shinyapps.io/cannabis-calculator.

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