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Thermal decarboxylation of acidic cannabinoids in *Cannabis* species: identification of transformed cannabinoids by UHPLC-Q/TOF–MS

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Abstract

Decarboxylation of cannabidiolic acid (CBDA) is an important step for efficient production of the active pharmaceutical component cannabidiol (CBD) in *Cannabis* species. Acidic cannabinoids (ACBs) can be easily transformed into neutral cannabinoids via loss of carbon dioxide when exposed to heat. During the thermal process, several transformed products including psychotropic Δ^9-tetrahydrocannabinol (Δ^9-THC) and its isomers were produced through decarboxylation, hydration, isomerization, and oxidation, as identified by ultra-high-performance liquid chromatography quadrupole/time-of-flight mass spectrometry (UHPLC-Q/TOF MS). Their identification was carried out using authenticated standards and interpreting the MS/MS fragmentations. To investigate thermal decarboxylation, CBDA was extracted and isolated from inflorescence of *Cannabis* by ultrasonication extraction and two-step column chromatography. To investigate the decarboxylation yield of isolated CBDA and ACBs in *Cannabis* extract, samples were examined over a range of reaction temperatures (110–130 °C) and times (5–60 min). Time profiles of CBDA degradation and CBD formation were obtained as functions of the reaction temperature. In particular, most of the CBDA was converted into CBD at 130 °C for 20 min; this CBD was partially transformed to psychotropic THC isomers via cyclization. In addition to THC isomers, cannabielsoin acid (CBEA) and cannabielsoin (CBE) were also observed as minor oxidative transformed products. Based on structural identification and profiling data, thermal transformation pathways of CBDA are plausibly suggested. The results of decarboxylation of ACBs will provide important information on production of neutral cannabinoids, especially CBD, in *Cannabis* plants and quality control of *Cannabis*-based products in pharmaceutical and cosmetic industries.

Keywords: *Cannabis* species, Cannabidiolic acid (CBDA), Thermal reaction, Psychotropic THC, UHPLC-Q/TOF–MS

Introduction

Recently, many studies have been conducted on medical applications of *Cannabis* species due to various pharmacological effects of cannabinoids. Cannabidiol (CBD) and tetrahydrocannabinol (THC) are well known cannabinoid components in *Cannabis* species. CBD in *Cannabis* species has been used for treatment of epileptic seizures, Parkinson's disease tremors, psychosis, and ulcerative colitis (Pauli et al. 2020). THC has been applied to treat nausea, appetite stimulation, and convulsions in patients with multiple sclerosis and glaucoma, but its use is strictly limited due to its psychoactive effects (Martínez et al. 2020). However, the amounts of acidic cannabinoids such as cannabidiol acid (CBDA) and tetrahydrocannabinol acid (THCA) are significantly higher than those

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of CBD and THC in *Cannabis* plants (Aizpurua-Olaizola et al. 2016). These acidic cannabinoids can be easily converted into their corresponding neutral cannabinoids by loss of CO_2 when exposed to high temperatures, UV exposure, or prolonged storage (Lewis et al. 2017; Tzimas et al. 2021).

Generally, CBDA and THCA of *Cannabis* species have some limitations in pharmaceutical applications. CBD and THC are delivered to the brain through the blood–brain barrier (BBB) and used to treat central nervous system diseases (Calapai et al. 2020; Russo and Marcu 2017), but CBDA and THCA can be ionized at physiological pH and do not pass well through the BBB (Anderson et al. 2019; Pajouhesh and Lenz 2005). In addition, acidic cannabinoids have low thermal stability and can be converted into other components in the pharmaceutical production process (Reason et al. 2022). Thus, for pharmaceutical applications of *Cannabis* species, it is necessary to convert the acidic cannabinoids into their corresponding neutral cannabinoids through a heat treatment process.

In previous studies (Moreno et al. 2020; Wang et al. 2016; Ryu et al. 2021), extracts of *Cannabis* were heat-treated or *Cannabis* plants were heat-dried at different temperatures for different lengths of time. In addition, differences between open and closed reactors were noticeable, where a larger total CBD loss was found in open reactors (Citti et al. 2018). The rate varies by individual cannabinoid. Decarboxylation of CBDA proceeded at a slower rate than that of THCA (Ryu et al. 2021). When the total content was compared, CBD was specifically decreased compared to total THC (Wang et al. 2016). The volatilization potentials of CBDA and CBD and the production of unknown compounds were suggested to explain this result (Moreno et al. 2020).

For identification and quantification of *cannabinoids*, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography (LC)–tandem mass spectrometry (MS/MS) have been the most popularly used tools (Gul et al. 2018; Macherone et al. 2020; Tran et al. 2022). GC–MS techniques have several advantages such as high separation capacity, high sensitivity and selectivity using selected ion monitoring, and a mass spectral library database. In particular, GC–MS can provide higher separation capability for similar structural analogues than LC–MS and reproducible spectral fragment patterns for analytes in electron ionization (EI) mode. However, a disadvantage of the GC-based method is that acid cannabinoids can be converted into their corresponding neutral cannabinoids through decarboxylation due to the high temperature of the GC system, producing ambiguous qualification and quantitation results (Lazarjani et al. 2020; Zivovinovic et al. 2018). Cannabinoids with carboxylic acid and/or hydroxyl groups produce poor chromatographic properties such as low peak intensity and peak tailing in GC analysis due to their reduced volatility. In order to apply the GC-based method, trimethylsilylation (TMS) should be introduced to enhance GC chromatographic properties as well as to protect against decarboxylation of acid cannabinoids in high-temperature injection pots and ovens (Ciolino et al. 2018).

LC–MS/MS techniques are sophisticated analytical tools owing to their high sensitivity and selectivity in quantification and qualification of cannabinoids using multiple-ion reaction monitoring (MRM) and product ion scanning modes, respectively. In particular, LC-high resolution (HR) MS was widely applied to identify the structures of cannabinoids extracted from *Cannabis* species and products transformed during the manufacturing process (Aizpurua-Olaizola et al. 2014; Citti et al. 2019; Ferrer et al. 2020; Chambers and Musah 2022). Generally, hybrid tandem Q/TOF high-resolution (HR) MS offers high sensitivity and selectivity in confirming the cannabinoids of *Cannabis*-based products. In particular, Q/TOF MS can be applied for confirmation of known and unknown cannabinoids because of its high mass resolving power ($\geq$ 20,000). Also, it can easily generate an HR-MS/MS spectrum with mass measurement errors less than 10 ppm for individual fragments. Thus, HR-common fragments for similar structured cannabinoids can be identified by interpretation of HR-MS/MS spectra.

In this study, the decarboxylation patterns of acidic cannabinoids including CBDA contained in *Cannabis* extract were examined under thermal reaction conditions. In addition, CBDA was isolated from *Cannabis* species to investigate its thermally transformed products. After thermal reaction of CBDA, transformed products including psychotropic THC isomers were identified by MS/MS fragmentation patterns and exact mass measurement. The time profiles of isolated CBDA and acid cannabinoids in *Cannabis* species were examined as a function of temperature to investigate the kinetic rates of CBDA. Based on the time profile data and identification results, the formation pathways of products from the thermal reaction of CBDA are proposed. The overall research concept of this study is briefly depicted in Fig. 1. These results will provide important guidelines for control of the manufacturing process in pharmaceutical applications of CBDA.

Experiments

Materials

Cannabis species samples [blending *Cannabis sativa,* Cherry Blossom hybrid] were received from Nongboomind Company (Seoul, Korea) under the permission of the

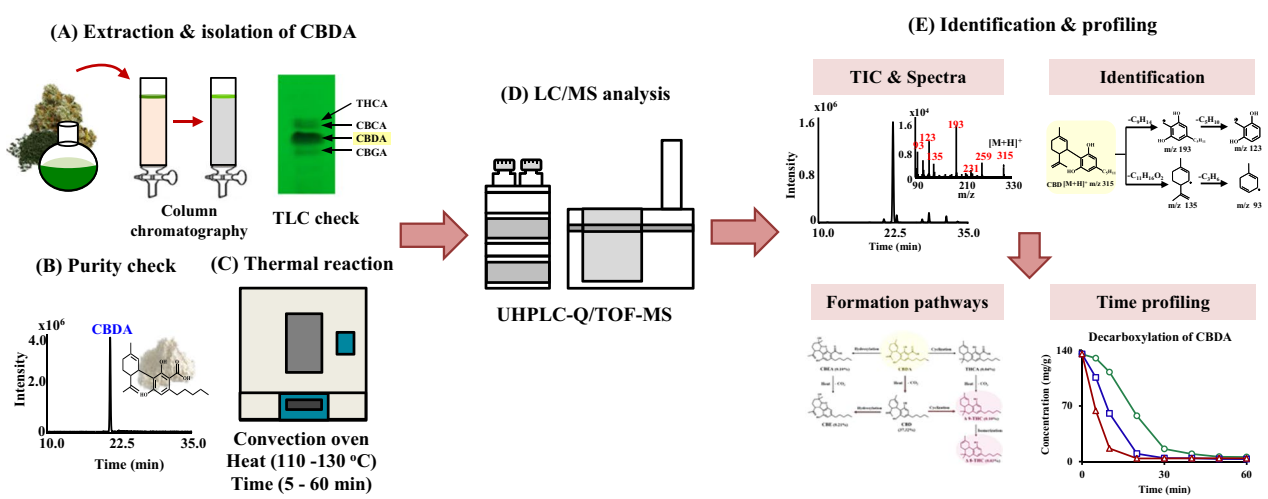

Fig. 1 Overall analytical procedure of thermal reaction for acidic cannabinoids. **A** extraction & isolation of CBDA, **B** purity check, **C** thermal reaction, **D** LC/MS analysis, and **E** identification & profiling

Ministry of Food and Drug Safety. These samples were air-dried in the dark at room temperature, ground using an electric grinder (Hanil, Seoul, Korea), and stored at −20 °C. Silica gel 60 (mesh size 0.040–0.063 mm) and Sephadex LH-20 (particle size 18–111 μm, dry) for isolation of CBDA were purchased from Merck (Darmstadt, Germany) and Cytiva (Uppsala, Sweden), respectively. TLC plates (silica gel 60 F_{254}) were purchased from Merck (Darmstadt, Germany). The natural convection oven used as the thermal reactor was supplied by JEIO TECH (ON-02GW, Seoul, Korea).

Reference standards including a mixture of 8 neutral cannabinoids [cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabichromene (CBC), cannabidivarin (CBDV), Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), tetrahydrocannabivarin (THCV)] and a 6-acid cannabinoid mixture (purity ≥ 98.5%) [cannabichromenic acid (CBCA), cannabidivarinic acid (CBDVA), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), tetrahydrocannabivarinic acid (THCVA), tetrahydrocannabinolic acid-A (THCA)] as 500 μg/mL ampoules were purchased from Cerilliant (Round Rock, TX, USA). Individual ampoules of 100 μg/mL solutions of isotopically labeled internal standards including Δ^9-THC-d_3, CBD-d_3, CBDA-d_3, and THCA-d_3 (purity ≥ 99.9%) were purchased from Cerilliant (Round Rock, TX, USA). These reference standards were diluted with methanol at analytical concentrations.

All reagents and organic solvents were extra pure and analytical grade for column chromatography and analysis, respectively. Ethyl acetate, methanol, and chloroform were purchased from Duksan Pure Chemicals (Ansan, Korea). Petroleum ether, ethyl alcohol, and dichloromethane were purchased from Daejung Chemical & Metal (Siheung, Korea). Analytical grade acetonitrile (ACN) and methanol (MeOH) were obtained from Honeywell (Morris Plains, NJ, USA). Formic acid (purity ≥ 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol used in the LC analysis were filtered through a 0.45-μm membrane filter and degassed for 10 min. De-ionized water was prepared using a Millipore Direct-Q3 purification system from the Millipore Corporation (Billerica, MA, USA), filtered through a 0.2-μm membrane filter, and degassed for 10 min prior to use. The trimethylsilyl (TMS) derivatization reagent *N,O-bis*(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (purity ≥ 99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used in the GC–MS analysis.

Isolation of CBDA from the extract of *Cannabis* inflorescence

Ten grams of ground *Cannabis* inflorescence was placed into 500 mL of ethanol in a 1 L beaker and sonicated for 60 min in an ultrasonicator bath (Branson, Denbury, CT, USA). After sonication and filtration (Grade 1 Qualitative Filter Paper, Whatman, Dassel, Germany), extract was concentrated using a rotary evaporator (EYELA, Tokyo, Japan), and 1.54 g of a dark green crude extract was obtained. The analytical procedure for extraction of cannabinoids and isolation of CBDA from *Cannabis* inflorescence is depicted in Fig. 2.

Two-step column chromatography was applied to isolate CBDA from the *Cannabis* inflorescence extract. Isolation of CBDA from *Cannabis* extract was carried out based on a previously reported method (Martinenghi

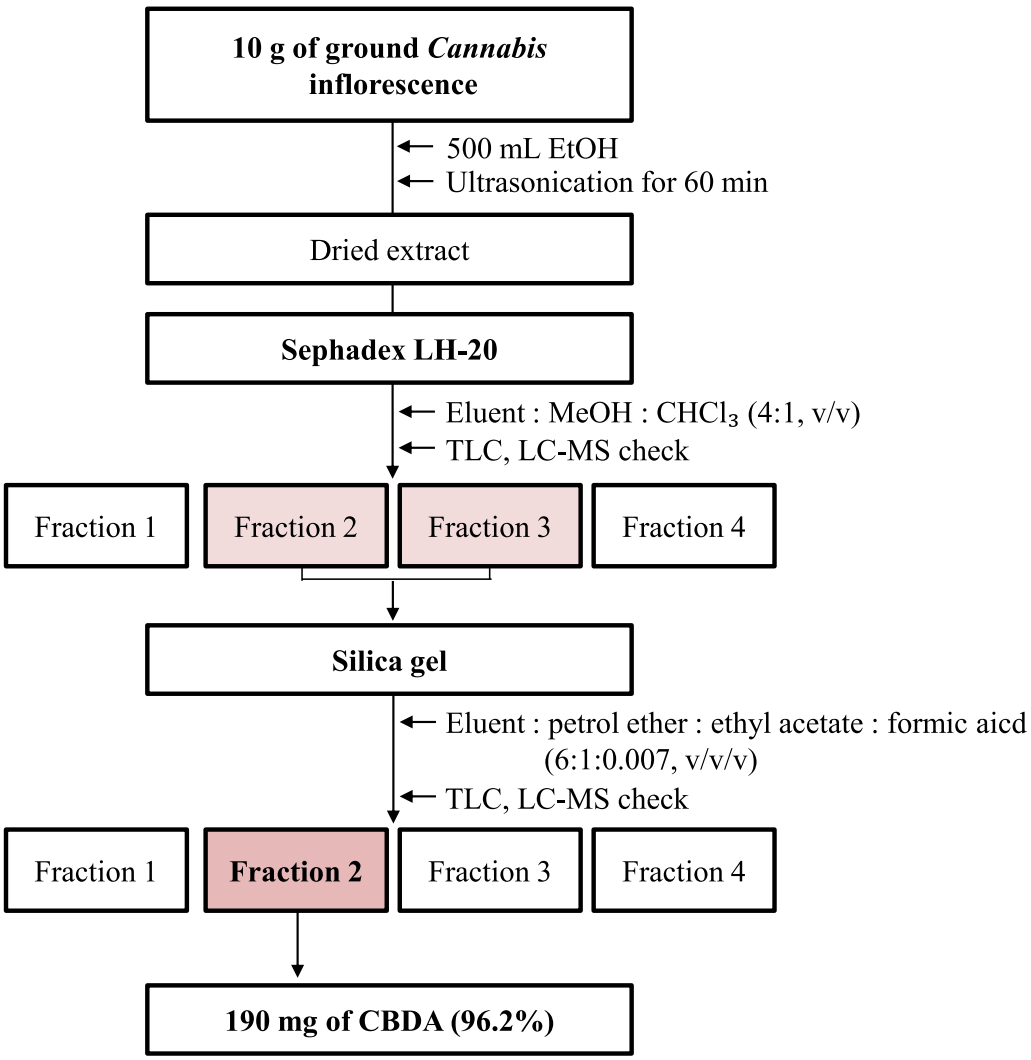

Fig. 2 Flow of isolation of CBDA from extract of Cannabis inflorescence

et al. 2020). Sephadex LH-20 was packed in slurry mode in a 50-cm column tube with a diameter of 3.5 cm. Dried extract residue (1.54 g) was re-dissolved in the eluent solvent, loaded into a Sephadex LH-20 column (35 mm × 500 mm), and eluted with $CHC1_3$-MeOH. Individual collections (each 7 mL) were monitored by TLC silica gel 60 F254 (Merck, Søborg, Denmark). A total of 4 fractions of eluents were collected in the TLC experiments. Fractions 2 and 3 were shown to include abundant CBDA and were combined. For further purification of the combined fractions 2 and 3, silica gel column chromatography (30 mm × 400 mm) was applied using petroleum ether–ethyl acetate–formic acid (6:1:0.007, v/v/v) as the eluent. In the 168–294 mL collected, approximately 190 mg of CBDA was effectively isolated. Its purity was checked in the LC–MS scan mode and was above 96%. The isolated CBDA was lyophilized in a freeze-dryer (Gyrozen, HyperCOOL HC3110, Seoul, Korea) at − 110 °C for 12 h to evaporate residual solvent, and CBDA powder was stored in an amber vial at − 20 °C. Isolated CBDA was confirmed by exact mass measurement, matching the retention time, and the MS/MS spectrum of the authentic standards in LC-Q/TOF MS analysis.

Decarboxylation of the extract of *Cannabis* inflorescence and isolated CBDA

The decarboxylation experiments of CBDA of *Cannabis* inflorescence extracts were performed on cherry blossom (CB). A *Cannabis* inflorescence sample (0.1 g) was dissolved in 5 mL of ethanol and extracted by sonication for 20 minutes. The *Cannabis* inflorescence extract was centrifuged at 6000 rpm for 5 min and then filtered using a 0.2-μm PTFE filter to remove

the solid parts of the extract. The stock solution of isolated CBDA was prepared in ethanol at a concentration of 1 mg/mL.

The thermal reaction was conducted in a convection oven for dried extract of *Cannabis* inflorescence and isolated CBDA in closed vials according to variations of temperature (110–130°C) and time (5–60 min). After the thermal reaction, the reactant sample was dissolved in ethanol and analyzed by UHPLC-Q/TOF MS.

LC-Q/TOF–MS conditions

Ultra-high-performance liquid chromatography (UHPLC) analysis was performed using an Agilent 1290 UHPLC system (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation was carried out on a Phenomenex Luna Omega polar C_{18} column (100 × 2.1 mm, 1.6 µm). Mobile phases A and B were 0.1% (v/v) formic acid in water and acetonitrile-methanol (9:1, v/v), respectively. The gradient elution mode was programmed as follows: 50% of mobile phase B for 0.0–15.0 min, 50–75% of B for 15–34.5 min, 75–95% of B for 34.5–35.0 min, 95% of B for 35–37 min, 95–50% of B for 37–37.5 min, and 50% B for 37.5–40 min. The flow rate, injection volume, and column temperature were set at 0.4 mL/min, 2 µL, and 40 °C, respectively.

All LC–MS and LC–MS/MS experiments were performed using a 6530 accurate mass quadrupole time-of-flight mass spectrometer instrument (Agilent Technologies, Santa Clara, CA, USA). This instrument was operated in the extended dynamic range of 2 GHz (m/z 1700 Th) in the high-resolution mode. Positive ions of analytes were generated using an electrospray ionization (ESI) source from Agilent Jet Stream Technology. The ESI source parameters included a superheated nitrogen sheath gas temperature of 350 °C and a flow of 11 L/min. The mass spectrometer conditions were a capillary voltage (Vcap) of 4000 V, nebulizer pressure of 45 psi, drying gas flow of 8 L/min, and a gas temperature of 300 °C. The fragmentor, skimmer, and octapole RF voltages were set at 120, 65, and 750 V, respectively. The mass scan ranges were m/z 90–400 for profiling of CBDA and CBD and m/z 100–1000 for purity analysis of isolated CBDA. Reference masses of m/z 121.050873 (purine) and 922.009798 (HP-0921) were used to calibrate the mass axis during analysis. The MS/MS experiments were performed at a fixed collision energy of 25 V. For MS/MS spectra of individual analytes, the $[M+H]^+$ ions were selected as precursor ions. The exact mass measurements for all of the MS and MS/MS data were controlled using MassHunter Qualitative and Quantitative Analysis software B.07.00 (Agilent Technologies, Santa Clara, CA, USA).

Results and discussion

Identification of thermally transformed cannabinoids of CBDA and *Cannabis* extract

To identify the products of CBD and *Cannabis* extracts transformed under thermal reaction conditions, individual reactant residues were analyzed by UHPLC-Q/TOF–MS. The typical total ion chromatograms (TICs) of the conversion products of CBDA obtained after the thermal reaction of *Cannabis* extracts are shown in Fig. 3. A total of 7 transformed products were observed for CBDA after thermal reaction at 130 °C for 30 min. Transformed products in the CBDA reaction solution were mainly formed through decarboxylation, hydration, and cyclization of CBD. As shown in Fig. 3A, after the thermal reaction of isolated CBDA, CBDA (peak 5) and CBD (peak 6) were detected as major components. Minor components such as CBEA (peak 3), CBE (peak 4), Δ^9-THC (peak 9), Δ^8-THC (peak 10), and THCA (peak 12) were also detected. These transformed products including CBDA were clearly identified by matching retention times (RTs) and mass spectra of authentic standards, except for CBEA and CBE. These two were tentatively identified by LC retention behavior (Luca et al. 2021), exact mass measurement, and interpretation of MS/MS spectral patterns due to the lack of authentic standards. The exact mass values of CBEA and CBE were within ± 3.5 ppm of their corresponding theoretical mass values. The MS/MS spectra and fragmentation patterns of CBE and CBEA were intensively interpreted and will be described in "ESI mass fragmentations of thermal transformation cannabinoids of CBDA and *Cannabis* extract" Section. Interestingly, THC isomers, known psychotropic substances, were observed at trace levels. Therefore, the occurrence of THC isomers should be carefully monitored and controlled during the thermal reaction of *Cannabis* and its derived products.

To investigate the transformed cannabinoid profiles, *Cannabis* extracts were analyzed and compared by LC/MS scans before and after the thermal reaction [Fig. 3B, C]. After thermal reaction of *Cannabis* extract at 130 °C for 30 min, acid cannabinoids of CBDA, THCA, CBDVA, and CBGA were almost fully converted into their corresponding neutral cannabinoids of CBD, THC, CBDV, and CBG, respectively, through decarboxylation. These compounds were identified by matching the RTs and mass spectra of authentic standards. Most of the CBDA in *Cannabis* extract was converted into CBD and expected to be transformed to Δ^9-THC, resulting in significant increase in its amount [peak 5 in Fig. 3B]. Decarboxylation of CBDA, THCA, CBDVA, and CBGA also readily converted these species into their corresponding neutral cannabinoids, exhibiting decarboxylation reaction yields of 97, 99, 93, and 98%

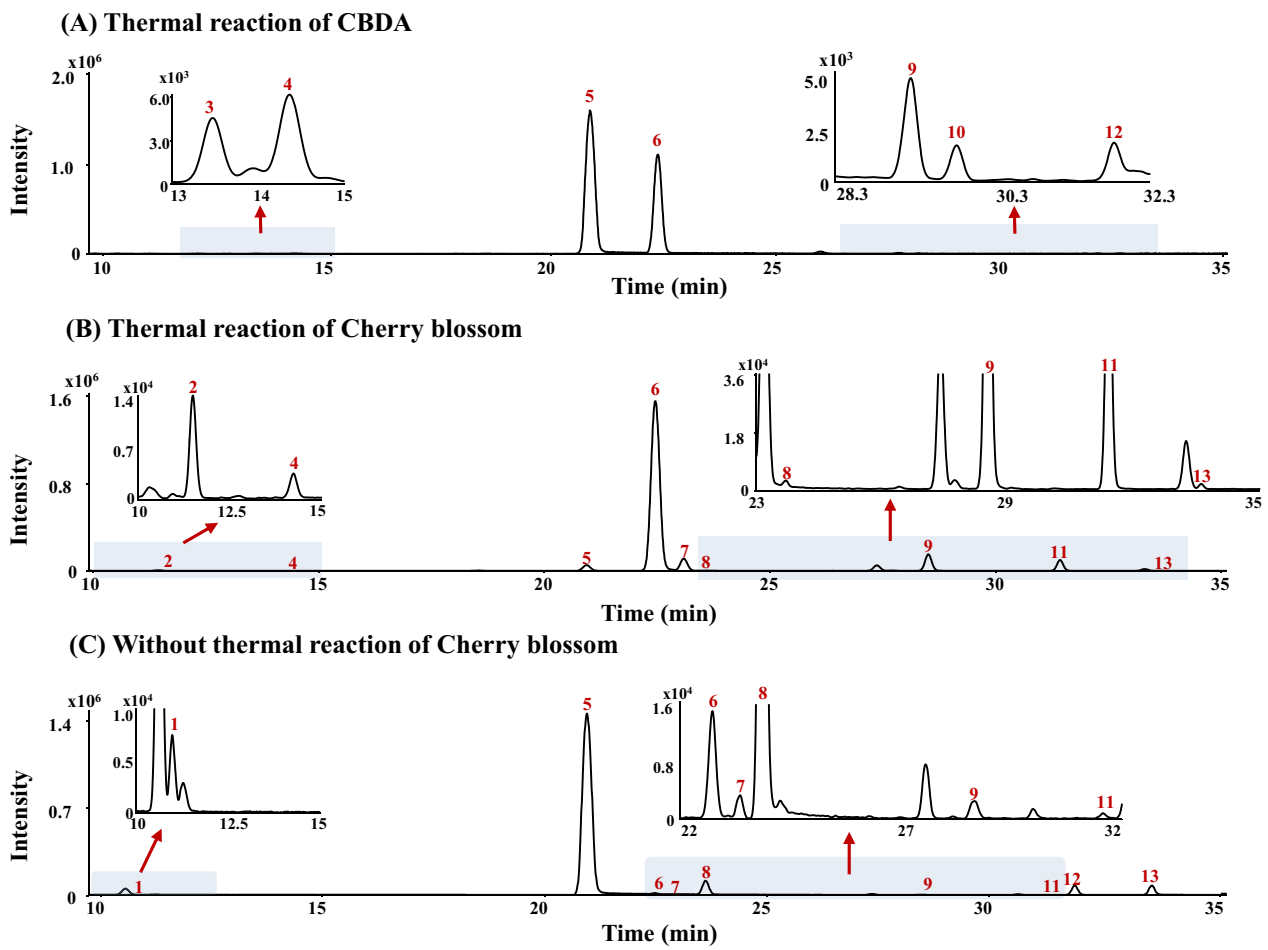

Fig. 3 TICs of **A** isolated CBDA, **B** extract of Cherry blossom after heat treatment at 130 °C for 30 min and **C** without heat treatment of Cherry blossom. Peak identities as follows: 1. CBDVA, 2. CBDV, 3. CBEA, 4. CBE, 5. CBDA, 6. CBD, 7. CBG, 8. CBGA, 9. Δ9-THC, 10. Δ8-THC, 11. CBC, 12. THCA, 13. CBCA

in *Cannabis* extract, respectively. However, about 43% of the isolated CBDA was decarboxylated, as shown in the TICs in Fig. 3A, indicating less frequent decarboxylation of CBDA than for *Cannabis* extract. This is consistent with the literature (Wang et al. 2016), where decarboxylation of CBDA readily occurred under thermal reaction of *Cannabis* extract but did not readily occur for isolated CBDA. This suggests that the matrix components in *Cannabis* extract play an important role as catalysts during the thermal reaction. Also, the formation yields of THCA and THC were less than 0.1% for isolated CBDA, indicating that CBDA was rarely converted into THCA and THC. On the other hand, no CBEA and CBE were observed without thermal treatment of *Cannabis* extract. These compounds are known to be formed under oxidative conditions of CBDA (Hanuš et al. 2016). This is thought to be generated by the reaction of a trace amount of oxygen in the closed vial with CBDA.

ESI mass fragmentations of thermal transformation cannabinoids of CBDA and *Cannabis* extract

All ESI–MS/MS spectra of thermal transformation products, neutral cannabinoids, and acidic cannabinoids obtained in this study are presented in Additional file 1: Fig. S1 and Additional file 1: Fig. S2. ESI mass spectra of cannabinoids exhibited protonated molecular ions $[M+H]^+$. The MS/MS spectra of acidic cannabinoids (CBDA, CBGA, CBCA, CBDVA, and THCA) can be characterized by the weak intensity of the $[M+H]^+$ ion and their common of $[M+H\text{-}H_2O]^+$, m/z 285, 261, and 233 ions and abundant m/z 219 ion. Their MS/MS spectra and fragmentation pathways are depicted in Additional file 1: Fig. S1 and Additional file 1: Scheme S1. These common fragment ions at m/z 285 and 261 might

be formed by losses of C_4H_8 (butene) and C_6H_8 (hexadiene) molecules from the $[M+H\text{-}H_2O]^+$ ion. Also, the abundant characteristic ion at m/z 219 may be produced by loss of C_3H_6 (propene) molecules accompanied by hydrogen migration at the unsaturated alkyl chain from the m/z 261 ion. Several fragment ions below m/z 177 may be formed by successive loss of CH_2 radicals at the alkane chain (C_5H_{11}) of the benzene ring from the m/z 219 ion.

For neutral cannabinoids [Additional file 1: Fig. S2], the weak $[M+H]^+$ ion and common characteristic ions $[M+H\text{-}C_4H_8]^+$ (A) and $[M+H\text{-}C_9H_{14}]^+$ (B) formed by cleavage of the substituted unsaturated group at the benzene ring were observed. Fragments A and B ions have a relatively stable benzyl cation. From alkane chain cleavage of A and B ions, other common ions such as $[A\text{-}C_2H_4]^+$, $[A\text{-}C_3H_6]^+$, $[B\text{-}C_2H_4]^+$, and $[B\text{-}C_5H_{10}]^+$ were also observed, as depicted in Additional file 1: Scheme S2. Below m/z 190, structures of common characteristic ions such as m/z 181, 153, 135, and 93 were suggested. ESI–MS/MS spectra of neutral cannabinoids can be characterized by the presence of these common ions. These fragmentation pathways can help to structurally elucidate new emerging cannabinoids in plants or transformed cannabinoids produced from thermal or chemical reactions of *Cannabis* species.

The MS/MS spectra of tentatively identified CBE and CBEA in this study are depicted in Fig. 4A, B. Structures of these substances with furan and cyclohexane rings are slightly different from those of other cannabinoids. Their MS/MS spectral patterns were complicated compared to those of other cannabinoids. For CBE, some of the common ions such as m/z 193, 165, 123, and 93 were the same as those of neutral cannabinoids. For CBEA, characteristic ions at m/z 357 and 339 formed by successive loss of H_2O molecules from the $[M+H]^+$ ion were observed, and the ions at m/z 231 and 219 were formed by the loss of C_8H_{12} and C_9H_{12} from the ion at m/z 339. The common ion of m/z 109 for CBEA and CBE was also observed. Based on the suggested fragmentation pathways of other acidic and neutral cannabinoids, fragmentation pathways of CBE and CBEA are suggested in Fig. 4C.

For further identification of CBE and CBEA, reactant residue was trimethylsilylated (TMS) with BSTFA and analyzed in GC/MS scan mode. As shown in Fig. 4D, E, CBE and CBEA were clearly detected as TMS derivatives in TIC, and their electron ionization (EI) mass spectra were obtained. Their molecular ions $[M^+]$ appeared at m/z 474 and 590, respectively, with weak intensities. The base peaks and abundant ions of the CBE-$(OTMS)_2$ and CBEA-$(OTMS)_3$ derivatives were commonly observed at m/z 108 $[C_8H_{12}]^+$ and m/z 130 $[C_3H_5OTMS]^+$, respectively, and these ions were formed by two bond cleavages of cyclohexane. Other characteristic ions were structurally assigned in their mass spectra, and the mass fragmentations are summarized in Fig. 4F. The ESI–MS/MS and GC/MS spectral data obtained in this study were complementary and useful for clear identification of acidic and neutral cannabinoids without authentic standards.

Time profiles of decarboxylation of CBDA during thermal reaction

In this study, the decarboxylation profiles of CBDA for *Cannabis* inflorescence extract and isolated CBDA were obtained at various thermal reaction temperatures. For quantification of the thermally transformed cannabinoids, isotope-labeled CBD-d3 and CBDA-d3 were used as internal standards. According to previous reports (Moreno et al. 2020; Ryu et al. 2021), CBDA was easily converted into CBD above 100 °C, but it was difficult to achieve sufficient decarboxylation of CBDA below 100 °C, even for 60 min. In this study, the temperature was adjusted among 110 °C, 120 °C, and 130 °C with various reaction times ranging from 5 to 60 min. Also, the thermal reaction was performed in a closed reactor to reduce the loss of neutral cannabinoids such as CBD and THC with relatively low boiling points. The time profiles of CBDA decarboxylation and CBD formation in the extract and isolated CBDA are shown in Fig. 5.

As shown in Fig. 5A, the amount of CBDA in *Cannabis* extract decreased radically when the temperature was increased. The reaction time needed to obtain maximum decarboxylation of CBDA decreased as the temperature increased. At 130 °C, the amount of CBDA in the extract decreased exponentially to 10 min, and no significant decrease was observed after 20 min. The maximum decarboxylation rate of CBDA in the extract was approximately 95% at 110 °C for 60 min, 97% at 120 °C for 50 min, and 97% at 130 °C for 20 min. This phenomenon clearly demonstrates that the decarboxylation reaction of CBDA is temperature dependent. On the other hand, the maximum formation rate of CBD was approximately 63% at 110 °C for 60 min, 64% at 120 °C for 50 min, and 65% at 130 °C for 20 min [Fig. 5A]. Compared to the decarboxylation efficiency of CBDA, the formation rate of CBD was low. This may be because some of the CBD was lost due to its relatively low boiling point even in the closed reaction vial, and CBDA was partly converted into other transformed products such THC isomers, THCA, CBEA, and CBE during the thermal reaction.

As shown in Fig. 5A, B, compared to the decarboxylation efficiency of CBDA in *Cannabis* extract, that of isolated CBDA was significantly lower. The decarboxylation efficiency of CBDA gradually increased as

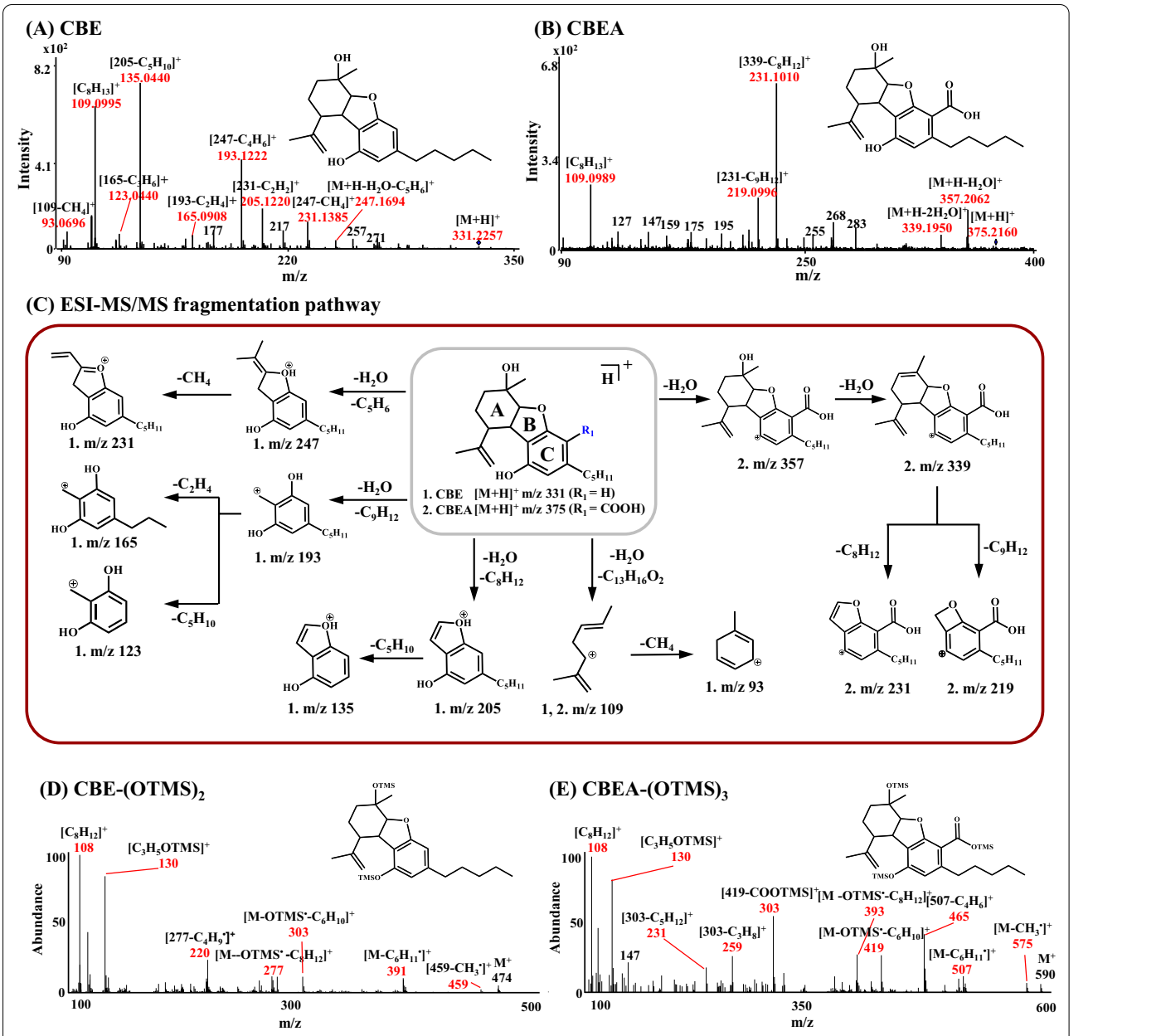

Fig. 4 ESI–MS/MS spectra of **A** CBE and **B** CBEA, **C** proposed ESI–MS/MS fragmentation pathways, EI mass spectra of **D** CBE and **E** CBEA, and **F** proposed EI mass fragmentation pathways

temperature and reaction time increased. At 130 °C for 60 min, the decarboxylation yield of isolated CBDA was about 52%. Also, the formation efficiency of CBD increased when temperature and reaction time increased. At 130 °C for 60 min, the formation yield of CBD was about 25%. This implies that the matrix of the extract significantly influences the decarboxylation of CBDA. In other words, various components in the matrix of the extract can significantly influence and accelerate the decarboxylation reaction of CBDA and other acidic cannabinoids. This phenomenon produced similar results in a previous study (Wang et al. 2016).

According to previous studies on the kinetics of CBDA decarboxylation (Citti et al. 2018), an Arrhenius plot for CBDA decarboxylation against 1/T (T: absolute temperature, Kelvin) was generated and is shown in Additional file 1: Fig. S3A. The linear equations and correlation coefficients were calculated using the least squares method

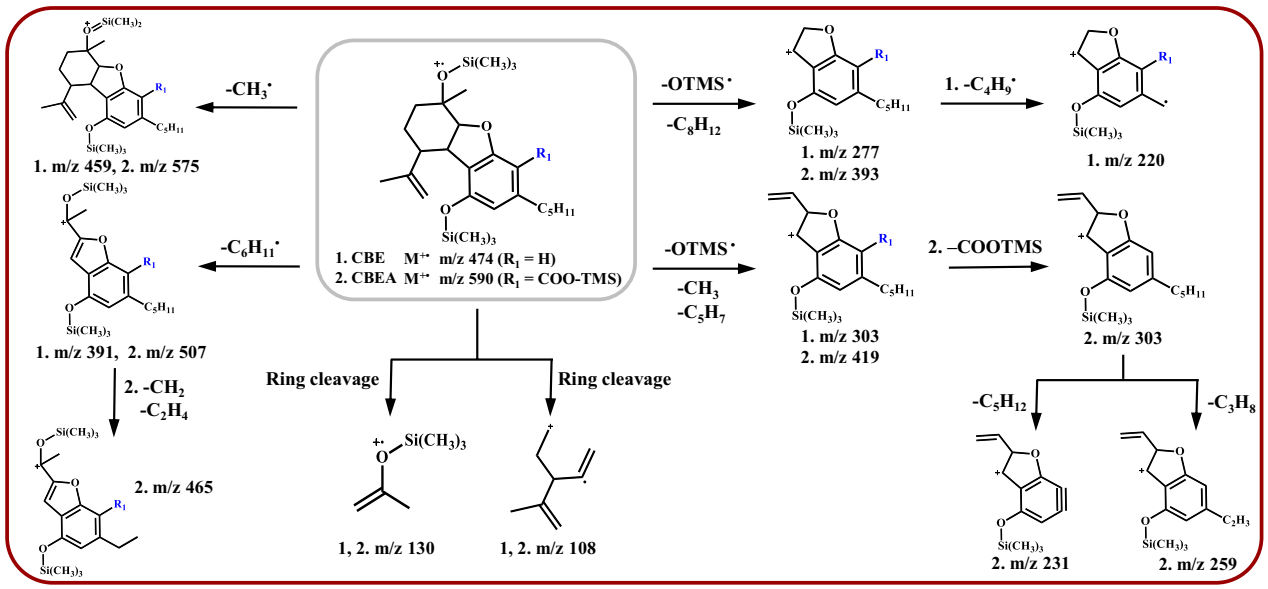

Fig. 4 continued

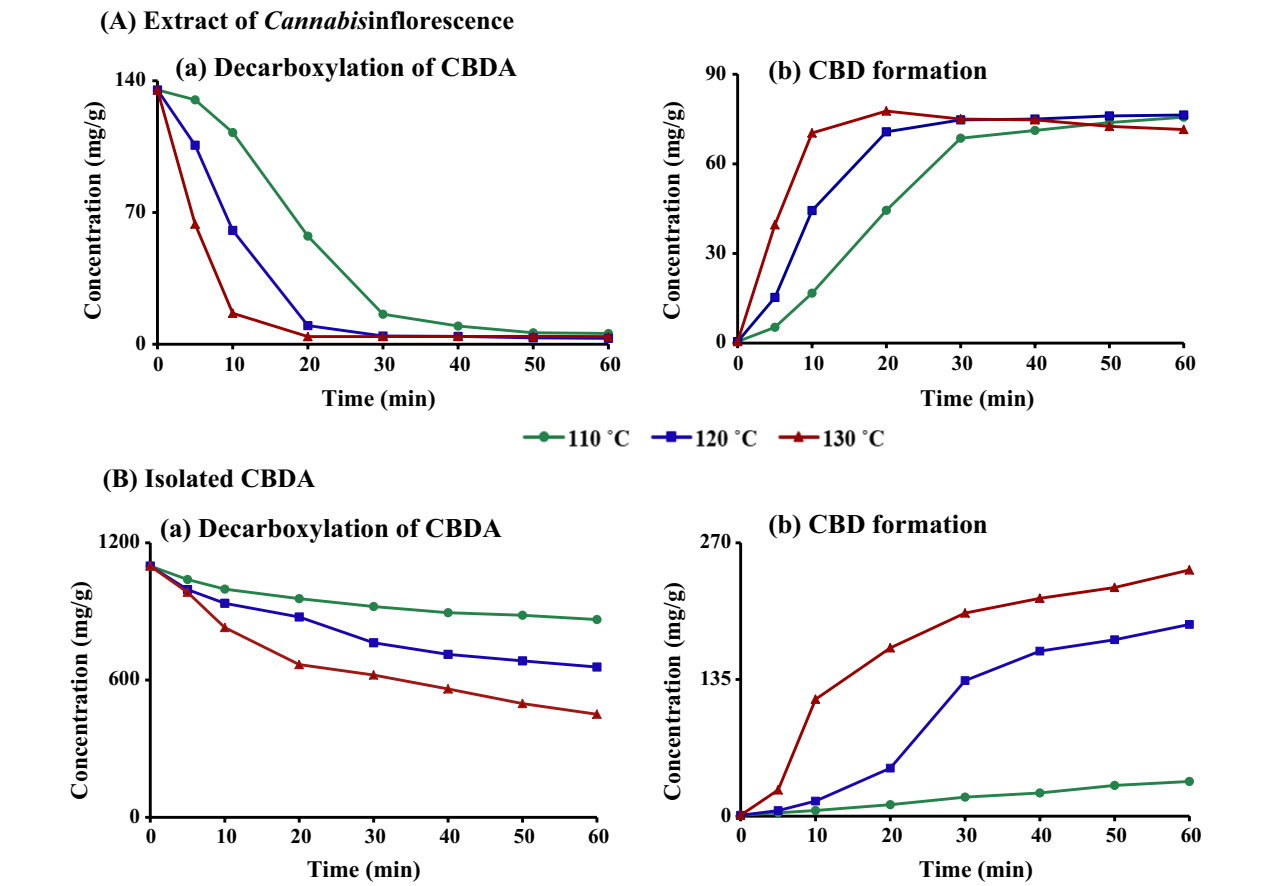

Fig. 5 Time profile of CBDA decarboxylation and CBD formation for **A** *Cannabis* extract and **B** isolated CBDA according to different temperature

(Nielsen et al. 2001). As expected, the kinetic rate constant (k) increased when the reaction temperature increased. Also, the reaction rates of CBDA decarboxylation with various reaction temperatures were determined in the graph of concentration (C) against reaction time, as illustrated in Additional file 1: Fig. S3B, C. The correlation coefficients of CBDA decarboxylation in extract are 0.9681, 9737, and 0.9829 at 110 °C, 120 °C, and 130 °C, respectively, indicating good linearity. Regarding CBDA decarboxylation, the reaction exhibited first-order kinetics within the reaction time range. Based on rate constants according to variation of reaction temperatures, the decarboxylation rate constants of the isolated CBDA were about tenfold less than those of CBDA in extract. It can explain that matrix components of *Cannabis* extract are expected to be acted as catalyzers in decarboxylation of CBDA, resulting in increasing rate constants. However, the decarboxylation rate constant of isolated CBDA mainly depends on reaction temperature. Thus, the reaction temperature above the threshold temperature (100 °C) is a key factor for decarboxylation of CBDA.

Based on the identified transformed products and time profile of CBDA decarboxylation, the transformation pathways during the thermal reaction of CBDA are suggested in Fig. 6. The formation of the major product, CBD, can be simply explained by loss of CO_2 molecules due to heat treatment. CBD can be partly converted into Δ^9-THC via intramolecular cyclization and then partly isomerized into Δ^8-THC (Hanuš et al. 2016). Although THC isomers were detected as minor components, they are potent psychoactive substances (Livne et al. 2022). Therefore, the formation of these compounds should be carefully monitored and controlled during processing of *Cannabis*-derived products.

Under thermal reaction of CBDA, CBEA and CBE were initially formed through epoxydation at the double bond of the cyclohexene ring to form an epoxy ring, followed by epoxy ring opening and intramolecular cyclization to form a furan ring. The formation mechanism of CBEA and CBE from CBDA is indicated in Scheme S3. It was reported that the thermal reaction of CBD in the presence of oxygen led to formation of CBE (Czégény et al. 2021). In this study, the amounts of CBEA and CBE significantly increased when the thermal reaction of CBDA was performed in an open reaction vial. Thus, conversion of CBDA into CBEA and

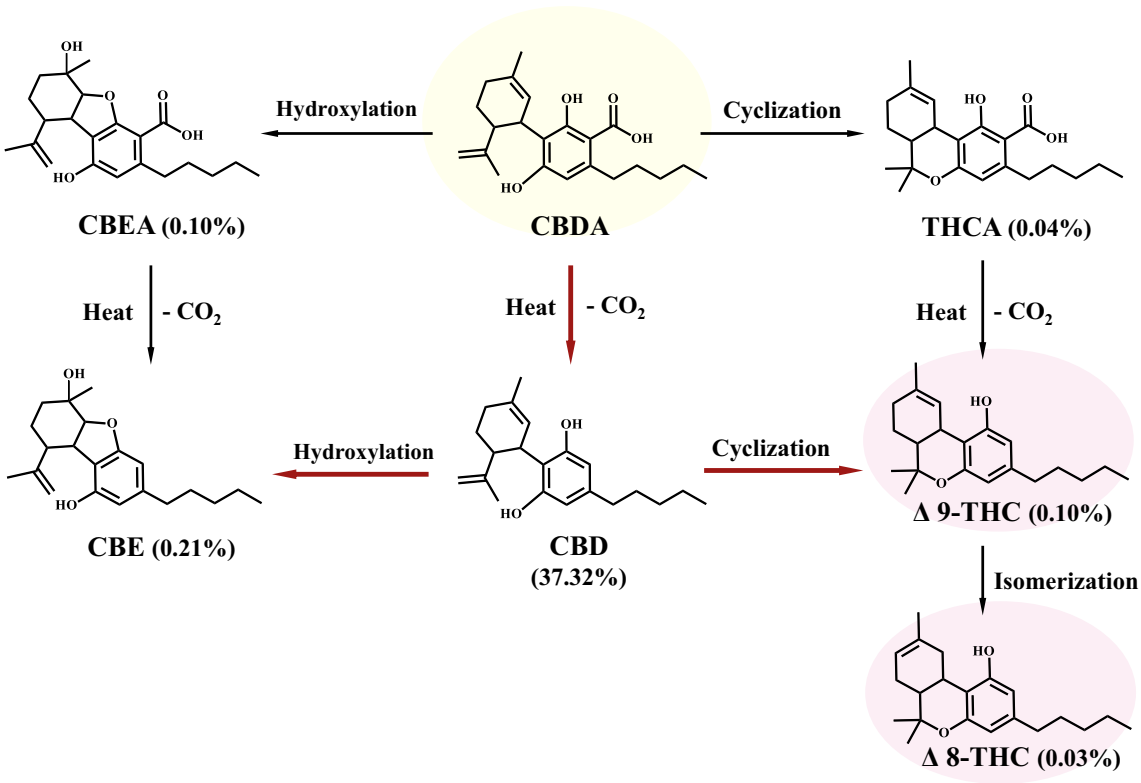

Fig. 6 Thermal transformation pathways of CBDA under thermal reaction at 130 °C for 30 min. Percentages are calculated using peak areas of total ion chromatogram

CBE could be greatly affected by both above threshold temperature and presence of oxygen.

Conclusion

In this study, chemical transformation of CBDA in a thermal reaction was investigated according to variations of reaction temperature and reaction time. UHPLC-Q/TOF–MS was successfully applied to obtain the mass spectra of the transformed products of CBDA and *Cannabis* extract under the thermal reaction of CBDA and *Cannabis* extracts. Based on the interpretation of mass spectra, several types of transformed products were clearly identified, and 13 cannabinoids in *Cannabis* extract, including psychoactive THC isomers, were observed. In this study, the formation of CBEA and CBE was observed in the thermal reaction of CBDA, and their formation mechanism was suggested. ESI–MS/MS fragmentations of cannabinoids were plausibly proposed based on structural characteristic ions. MS/MS fragmentations are useful for identification of related cannabinoids and newly transformed cannabinoids during thermal treatment of *Cannabis* to produce various molecules that can be used as dietary supplements, drugs, and cosmetic products.

Based on identification of the transformed products and time profile data of CBDA, the formation pathways and formation mechanisms under thermal reaction conditions are suggested. The formation pathways and mechanisms of transformed products are pertinent to safety management and quality guidelines in the processing of *Cannabis*-based products.

Abbreviations

ACBs: Acidic cannabinoids; BBB: Blood–brain barrier; CBC: Cannabichromene; CBCA: Cannabichromenic acid; CBD: Cannabidiol; CBDV: Cannabidivarin; CBDA: Cannabidiolic acid; CBDVA: Cannabidivarinic acid; CBE: Cannabielsoin; CBEA: Cannabielsoin acid; CBG: Cannabigerol; CBGA: Cannabigerolic acid; EI: Electron ionization; ESI: Electrospray ionization; GC–MS: Gas chromatography–mass spectrometry; HR: High resolution; LC–MS/MS: Liquid chromatography–tandem mass spectrometry; MRM: Multiple-ion reaction monitoring; THCA: Tetrahydrocannabinolic acid; TICs: Total ion chromatograms; TMS: Trimethylsilylation; UHPLC-Q/TOF MS: Ultra-high-performance liquid chromatography quadrupole/time-of-flight mass spectrometry; Δ^9-THC: Delta-9-tetrahydrocannabinol; Δ^8-THC: Delta-8-tetrahydrocannabinol.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-022-00351-4.

Additional file1. **Fig. S1**. ESI MS/MS spectra of acidic cannabinoids extracted from Cannabis extract by UHPLC-Q/TOF-MS. **Scheme S1**. Proposed ESI MS/MS fragmentation pathways of acidic cannabinoids. **Fig. S2**. ESI MS/MS spectra of neutral cannabinoids extracted from Cannabis extract by UHPLC-Q/TOF-MS. **Scheme S2**. Proposed ESI mass fragmentation pathways of neutral cannabinoids. **Fig. S3**.**A** Arrhenius plots for CBDA decarboxylation in extract and decarboxylation kinetics of**B** CBDA inextract and**C** isolated CBDA. **Scheme S3**. Formation mechanism of CBE and CBEA during heat treatment of CBDA.

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Author contributions

All authors read and approved the final manuscript.

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Availability of data and materials

The datasets of this manuscript are available upon request.

Declarations

Competing interests

There are no conflicts of interest to declare.

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References

Aizpurua-Olaizola O, Omar J, Navarro P, Olivares M, Etxebarria N, Usobiaga A. Identification and quantification of cannabinoids in *Cannabis sativa* L. plants by high performance liquid chromatography-mass spectrometry. Anal Bioanal Chem. 2014;406:7549–60. https://doi.org/10.1007/s00216-014-8177-x.

Aizpurua-Olaizola O, Soydaner U, Öztürk E, Schibano D, Simsir Y, Navarro P, Etxebarria N, Usobiaga A. Evolution of the cannabinoid and terpene content during the growth of *Cannabis sativa* plants from different chemotypes. J Nat Prod. 2016;79(2):324–31. https://doi.org/10.1021/acs.jnatprod.5b00949.

Anderson LL, Low IK, Banister SD, McGregor IS, Arnold JC. Pharmacokinetics of phytocannabinoid acids and anticonvulsant effect of cannabidiolic acid in a mouse model of dravet syndrome. J Nat Prod. 2019;82(11):3047–55. https://doi.org/10.1021/acs.jnatprod.9b00600.

Calapai F, Cardia L, Sorbara EE, Navarra M, Gangemi S, Calapai G, Mannucci C. Cannabinoids, blood-brain barrier, and brain disposition. Pharmaceutics. 2020;12(3):265. https://doi.org/10.3390/pharmaceutics12030265.

Chambers MI, Musah RA. DART-HRMS as a triage approach for the rapid analysis of cannabinoid-infused edible matrices, personal-care products and *Cannabis sativa* hemp plant material. Forensic Chem. 2022;27: 100382. https://doi.org/10.1016/j.forc.2021.100382.

Ciolino LA, Ranieri TL, Taylor AM. Commercial cannabis consumer products part 1: GC–MS qualitative analysis of cannabis cannabinoids. Forensic Sci Int. 2018;289:429–37. https://doi.org/10.1016/j.forsciint.2018.05.032.

Citti C, Pacchetti B, Vandelli MA, Forni F, Cannazza G. Analysis of cannabinoids in commercial hemp seed oil and decarboxylation kinetics studies of cannabidiolic acid (CBDA). J Pharm Biomed Anal. 2018;149:532–40. https://doi.org/10.1016/j.jpba.2017.11.044.

Citti C, Linciano P, Panseri S, Vezzalini F, Forni F, Vandelli MA, Cannazza G. Cannabinoid profiling of hemp seed oil by liquid chromatography coupled to high-resolution mass spectrometry. Front Plant Sci. 2019;10:120. https://doi.org/10.3389/fpls.2019.00120.

Czégény Z, Nagy G, Babinszki B, Bajtel Á, Sebestyén Z, Kiss T, Csupor-Löffler B, Tóth B, Csupor D. CBD, a precursor of THC in e-cigarettes. Sci Rep. 2021;11:8951. https://doi.org/10.1038/s41598-021-88389-z.

Ferrer I. Chapter fourteen - analyses of cannabinoids in hemp oils by LC/Q-TOF-MS. Compr Anal Chem. 2020;90:415–52. https://doi.org/10.1016/bs.coac.2020.04.014.

Gul W, Gul SW, Chandra S, Lata H, Ibrahim EA, ElSohly MA. Detection and quantification of cannabinoids in extracts of cannabis sativa roots using LC-MS/MS. Planta Med. 2018;84(04):267–71. https://doi.org/10.1055/s-0044-100798.

Hanuš LO, Meyer SM, Muñoz E, Taglialatela-Scafatid O, Appendino G. Phytocannabinoids: a unified critical inventory. Nat Prod Rep. 2016;33:1357–92. https://doi.org/10.1039/C6NP00074F.

Kumar P, Mahato DK, Kamle M, Borah R, Sharma B, Pandhi S, Tripathi V, Yadav HS, Devi S, Patil U, Xiao J, Mishra AK. Pharmacological properties, therapeutic potential, and legal status of *Cannabis sativa* L.: an overview. Phytother Res. 2021;35:6010–29. https://doi.org/10.1002/ptr.7213.

Lazarjani PM, Torres S, Hooker T, Fowlie C, Young O, Seyfoddin A. Methods for quantification of cannabinoids: a narrative review. J Cannabis Res. 2020;2:35. https://doi.org/10.1186/s42238-020-00040-2.

Lewis MM, Yang Y, Wasilewski E, Clarke HA, Kotra LP. Chemical profiling of medical cannabis extracts. ACS Omega. 2017;2(9):6091–103. https://doi.org/10.1021/acsomega.7b00996.

Livne O, Budney A, Borodovsky J, Walsh C, Shmulewitz D, Fink DS, Struble CA, Habib M, Aharonovich E, Hasin DS. Delta-8 THC use in US adults: sociodemographic characteristics and correlates. Addict Behav. 2022;133:107374. https://doi.org/10.1016/j.addbeh.2022.107374.

Luca SV, Braumann L, Gerigk M, Frank O, Minceva M. Separation of minor cannabinoids from hemp extract with trapping multiple dual mode liquid-liquid chromatography. J Chromatogr A. 2021;1658: 462608. https://doi.org/10.1016/j.chroma.2021.462608.

Macherone A. A brief review of derivatization chemistries for the analysis of cannabinoids using GC–MS. Cannabis Sci Tech. 2020;3(7):42–8.

Martinenghi LD, Jønsson R, Lund T, Jenssen H. Isolation, purification, and antimicrobial characterization of cannabidiolic acid and cannabidiol from *Cannabis sativa* L. Biomolecules. 2020;10(6):900. https://doi.org/10.3390/biom10060900.

Martínez V, Iriondo De-Hond A, Borrelli F, Capasso R, Del Castillo MD, Abalo R. Cannabidiol and other non-psychoactive cannabinoids for prevention and treatment of gastrointestinal disorders: Useful nutraceuticals? Int J Mol Sci. 2020;21:3067.

Moreno T, Dyer P, Tallon S. Cannabinoid decarboxylation: a comparative kinetic study. Ind Eng Chem Res. 2020;59:20307–15. https://doi.org/10.1021/acs.iecr.0c03791.

Nielsen L. Evaluation of measurements by the method of least squares. Algorithms For Approximation IV. ADP013728

Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. Neurotherapeutics. 2005;2:541–53. https://doi.org/10.1602/neurorx.2.4.541.

Pauli CS, Conroy M, Vanden Heuvel BD, Park S-H. Cannabidiol drugs clinical trial outcomes and adverse effects. Front Pharmacol. 2020;11:63. https://doi.org/10.3389/fphar.2020.00063.

Reason DA, Grainger MN, Lane JR. Optimization of the decarboxylation of cannabis for commercial applications. Ind Eng Chem Res. 2022;61:7823–32. https://doi.org/10.1021/acs.iecr.2c00826.

Russo EB, Marcu J. Cannabis pharmacology: the usual suspects and a few promising leads. Adv Pharm. 2017;80:67–134. https://doi.org/10.1016/bs.apha.2017.03.004.

Ryu BR, Islam MJ, Azad MOK, Go E-J, Rahman MH, Rana MS, Lim Y-S, Lim J-D. Conversion Characteristics of some major cannabinoids from hemp (*Cannabis sativa* L.) raw materials by new rapid simultaneous analysis method. Molecules. 2021;26(14):4113. https://doi.org/10.3390/molecules26144113.

Tran J, Elkins AC, Spangenberg GC, Rochfort SJ. High-throughput quantitation of cannabinoids by liquid chromatography triple-quadrupole mass spectrometry. Molecules. 2022;27(3):742. https://doi.org/10.3390/molecules27030742.

Tzimas PS, Petrakis EA, Halabalaki M, Skaltsounis LA. Effective determination of the principal non-psychoactive cannabinoids in fiber-type *Cannabis sativa* L. by UPLC-PDA following a comprehensive design and optimization of extraction methodology. Anal Chim Acta. 2021;1150:338200. https://doi.org/10.1016/j.aca.2021.338200.

Wang M, Wang YH, Avula B, Radwan MM, Wanas AS, van Antwerp J, Parcher JF, ElSohly MA, Khan IA. Decarboxylation study of acidic cannabinoids: a novel approach using ultra-high-performance supercritical fluid chromatography/photodiode array-mass spectrometry. Cannabis Cannabinoid Res. 2016;1(1):262–71. https://doi.org/10.1089/can.2016.0020.

Zivovinovic S, Alder R, Allenspach MD, Steuer C. Determination of cannabinoids in *Cannabis sativa* L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection. J Anal Sci Technol. 2018;9:27. https://doi.org/10.1186/s40543-018-0159-8.

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