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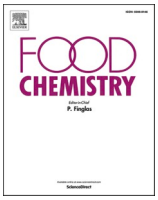

Transformation of Cannabidiol (CBD) during its high-temperature extraction: Studies involving model systems, hemp and functional foods containing CBD

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ABSTRACT

Most approaches to plants and foodstuffs analysis involve the application of liquid extraction methods for the isolation of their components. The present paper shows and discusses the transformations that CBD may undergo during its high-temperature extraction with methanol, dichloromethane, ethyl acetate and hexane from hemp and CBD containing commercially available functional foods. According to the performed research, CBD can transform not only into cannabinoids, which are hemp metabolites (i.e. Δ8/Δ9-THC, CBN), but also into other previously unknown derivatives, the number and quantity of which depend on the type of the extrahent and the oxygen content in the extraction system. In the case of each solvent used, the CBD transformation degree is strongly affected by the humidity of the extracted hemp. The results presented in this work are important both for hemp analysis, including the research dealing with hemp metabolism, but also for functional food containing CBD.

1. Introduction

Cannabidiol (CBD), 2-[(1*R*,6*R*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-2-enyl]-5-pentylbenzene-1,3-diol, one of the most abundant plant cannabinoids, arouses great interest among doctors and pharmacists due to its biological activity suggesting a potential use in medical therapies. Although the treatment of epileptic syndromes and some other central motor disorders is most frequently mentioned, positive effects of CBD reported in the literature are wider. Its potential use is being considered in the treatment of immune dysfunctions, diabetes, cancer and addictive behavior (Amin & Ali, 2019; Caffarel, Andradas, Pérez-Gómez, Guzmán, & Sánchez, 2012; Devinsky et al., 2014; Lo Faro et al., 2022; Marchioni, Vieira, Miller Crotti, Crippa, & Costa Queiroz, 2020; Massi, Solinas, Cinquina, & Parolaro, 2013; Mechoulam & Hanuš, 2002; Palmieri, Laurino, & Vadalà, 2017; Pertwee, 2005; Rock et al., 2012). Due to CBD ability to interact with various neurochemical pathways associated with reducing addiction and withdrawal syndromes, the clinical interest has involved recently the use of this cannabinoid, for example, in tackling the opioid crisis (De Aquino, Bahji, & D'Souza, 2022) or for the attenuation of unwanted side-effects of Δ9-THC used as a medicine. There are also reports dealing with positive outcomes in patients with schizophrenia (McGuire et al., 2018), while preclinical studies demonstrate anti-nausea and analgesic effects of CBD. Considerable interest in the bioactive properties of CBD resulting from a number of preclinical and clinical studies and observations, as well as a marked increase in the use of dietary supplements containing CBD in the human self-treating process (Vlad et al., 2021), stimulates the development of reliable and sensitive analytical procedures of its quantitation not only in blood/plasma samples but also in its plant sources and food products.

The analysis of CBD or other plant metabolites in plant materials, cosmetics, diet supplements and food products usually demands the application of a sample preparation method that allows for full isolation of the analyzed substances from examined matrices. Extraction methods are usually involved for this purpose (Aili et al., 2024; Bartončíková, Lapčíková, Lapčík, & Valenta, 2023; Bitwell, Indra, Luke, & Kakoma, 2023; Christinat & Mottier, 2020; Hsu et al., 2021; Lazarjani, Young, Kebede, & Seyfoddin, 2021; Myers, Herrington, Hamrah, & Anderson, 2021; Pisciottano, Guadagnuolo, Soprano, Esposito, & Gallo, 2021). Recently, extraction methods supported by pressure, ultrasound and microwaves have been increasingly used, which, apart from many important positive effects, allow to reach a high recovery of analytes during their isolation from examined material. Pressure-assisted extraction (PLE) deserves special attention, not only because of its high extraction efficiency resulting from the possibility of performing

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the extraction process at temperatures higher than the boiling temperature of the extracting solvent, but also because of its isolation selectivity, easily changeable by selecting the extrahent type and operating parameters of the extraction process (Moret et al., 2014), i.e. temperature and pressure.

It should be noted here that the conditions prevailing in the PLE extraction vessel may favor the decomposition or transformation of the isolated compound, and even its reaction with the extractant used. This may lead not only to an incorrect quantitative assessment of the tested compound, but also to an incorrect assessment of the qualitative composition of the extracted material. In the case of CBD analysis in the above-mentioned matrices, methanol is most often used. This work presents the results of CBD transformation in the case of its high-temperature extraction with methanol (MeOH) and also with solvents generally recognized as safe (GRAS) - dichloromethane (DCM), ethyl acetate (EtOAc), and hexane (Hex) – which are just as often used in PLE. CBD transformation during high-temperature extraction were studied not only on model samples, but also on hemp and selected food products containing CBD such as chips, chocolate, coffee, cookies, jelly candy, lollipops and hemp tea. These studies are justified because CBD, due to the presence of double bonds and hydroxyl groups in the molecule, may undergo addition, esterification/transesterification or substitution reactions (Dawidowicz, Dybowski, Typek, Rombel, & Holowinski, 2023; Holowinski, Typek, Dawidowicz, Rombel, & Dybowski, 2022; McMurry, 2007; Typek, Holowinski, Dawidowicz, Dybowski, & Rombel, 2023). Its oxidation to quinone forms has also been reported (; Caprioglio, Mattoteia, Taglialatela-Scafati, Muñoz, & Appendino, 2021; Kogan, Peters, & Mechoula, 2021; Trac, Keck, & Deweese, 2021). Very high temperatures during the extraction process may also lead to polymerization of the CBD molecule. Besides, in an acidic environment, CBD readily undergoes transformation to delta-9-THC and indirectly to delta-8-THC (Buijs, 2023; Dybowski, Dawidowicz, Typek, & Rombel, 2020; Watanabe et al., 2007).

2. Experimental

2.1. Materials

The CRM standard solutions of cannabidiol (CBD), tetrahydrocannabinol (Δ9-THC), tetrahydrocannabinol (Δ8-THC), cannabinol (CBN), all of 1.0 mg/mL in methanol (Cerilliant), acetonitrile (HPLC grade), and formic acid were purchased from Merck (Darmstadt, Germany). Methanol (MeOH) dichloromethane (DCM), ethyl acetate (EtOAc) and hexane (Hex) came from Avantor Performance Materials Poland (Gliwice, Poland). CBD crystal (> 99.5 %) was a gift from CannLAB (Kraków, Poland). Hemp plant, Carmagnola, was the gifts from local growers in the Lublin district (Poland). Cannabis Lollypop (CBD Alchemy, Barcelona, Spain), CBD cannabis cookies (Cannabis Beakehouse, Bari, Italy), Cannabis chips (Cannabis Beakehouse, Bari, Italy), CBD Infused Dark Roast Colombian Coffee (The CBD store, Galway, Ireland) and Cannabis tea with CBD (Konopny swiat, Otovice, Czech) were bought in Internet shop. Greenout Sweet bears (jelly candy) and Greenout Choco drops were from Dutch Therapy (Słupsk, Poland). Deionized water was purified by the Milli-Q system (Millipore Sigma, Bedford, MA, USA).

2.2. Hemp drying

Dried Carmagnola hemp was used in experiments. A two-step drying process was used: hemp heating at 105 °C to reach its constant mass subsequently heated at 115 °C for 1 h. Before experiments, the dried plant material was homogenized and examined to estimate the concentration of the main hemp cannabinoids and water content.

2.3. Hemp hydration

To estimate the influence of water content in hemp on the CBD degradation and formation of its derivatives, hemp samples containing different amount of water were used. The samples were prepared adding proper amounts of water to carefully weighed samples of dried hemp.

2.4. Determination of CBD concentration in hemp and food products

30 mg of dried hemp or food product (chips, chocolate, coffee, cookies, jelly candy, lollipops and hemp tea) was extracted with 1 mL of methanol for 15 min using ultrasonic bath (Sonic-6, Polsonic, Poland) working with 40 kHz frequency and 540 W power. After that the separated by centrifugation liquid phase was subjected to GC–MS analysis. Concentration of CBD in individual matrices were estimated by relating its chromatographic response to the calibration curve prepared with the use of CBD standard.

2.5. Sample treatment

Two groups of experiments should be distinguished in this work: experiments with CBD standard and hemp, and experiments with CBD containing foodstuffs.

2.6. Study with CBD and hemp samples

The research consisted of the analysis of:

- CBD standard solutions and
- hemp suspensions

prepared in various solvents exposed to high temperatures (100, 125, 150, 175 or 200 °C) for various periods of time (10, 20 or 30 min). Custom-made tightly closed stainless steel vessel (10 mL) filled completely or partially (3/4, 1/2 and/or 1/4 of its volume) with one of the above-mentioned samples were used. In the case of a partially filled vessel, the remaining part was air and solvent vapors. To prepare CBD solutions and hemp suspensions, the following solvents were used: MeOH, MeOH with water addition (0.25, 0.50, 0.75, 1.00, 2.50 and 5.00 %), DCM, EtOAc or Hex.

In all the experiments, the concentration of CBD was 1 mg per 1 mL of its solution or 1 mg per 1 mL of hemp suspension. For clarity, in the case of hemp, a certain amount of it was introduced into the high-pressure vessel so that 1 mL of its suspension contained 1 mg of CBD. The obtained high-temperature extracts were analyzed by GC–MS and LC-MS and solid particles present in them were removed by centrifugation before analyses.

2.7. Low- and high-temperature extraction of CBD containing foodstuffs

The research consisted of the analysis of methanolic extracts of foodstuffs with CBD, which were obtained in 20 min extraction processes at 25 °C (low-temperature extractions) and 175 °C (high-temperature extractions). Low-temperature extractions were assisted by ultrasounds using ultrasonic bath (Sonic-6, Polsonic, Poland) working with 40 kHz frequency and 540 W power). High-temperature extractions were performed in tightly closed stainless steel vessel (see description in previous subsection - *Study with CBD and hemp samples*). To better compare extraction results obtained for foodstuff products with those obtained for CBD standard the extraction vessels contained such amount of foodstuff sample to reach CBD concentration ca. 1 mg/mL. Before extraction the foodstuffs were shredded. The obtained high-temperature foodstuffs extracts were centrifuged and analyzed by and LC-MS.

2.8. GC–MS measurements

A gas chromatograph hyphenated with a triple quadruple tandem mass spectrometer detector (GCMS-TQ8040; Shimadzu, Kyoto, Japan) was used. GC–MS conditions were as follows: capillary column - Zebron

ZB5-MSi (30 m × 0.25 mm i.d., 0.25 μm film thickness; Phenomenex, Torrance, CA, USA); carrier gas - helium (grade 5.0); flow rate 1.0 mL/min; splitless/split injection mode (sampling time 1.00 min); injector temperature 310 °C; injection volume 1 μL; temperature program - initial temperature 60 °C held for 3 min and then increased to 300 °C at the rate of 12 °C/min. The final temperature was held for 12 min. Mass spectrometer parameters: normalized electron energy of 70 eV; ion source temperature 185 °C. For qualitative estimation the full Q3-SCAN mode with range 45–400 *m/z* was employed.

2.9. LC-MS measurements

LC-MS system composed of an UHPLC chromatograph (UltiMate 3000, Dionex, Sunnyvale, CA, USA) and a linear trap quadrupole-Orbitrap mass spectrometer (LTQ-Orbitrap Velos, Thermo Fisher Scientific, San Jose, CA) were applied for the chromatographic analyses of the examined extracts. ESI ionization source operating in the negative polarization mode at needle potential equal to 4.5 kV was employed. Nitrogen (>99.98 %) was used as sheath gas (at 40 arbitrary units), auxiliary gas (at 10 arbitrary units) and sweep gas (at 10 arbitrary units). Capillary temperature was 320 °C. The scan cycle used a full-scan event at the resolution of 60,000. Chromatographic separations were performed on a Gemini C18 column (4.6 × 100 mm, 3 μm; Phenomenex, USA). The mobile phase components were: A – 25 mM formic acid in water, and B - 25 mM formic acid in acetonitrile. The gradient program started at 25 % B, increasing to 70 % for 45 min, next from 70 % to 100 % B for 5 min, and ended with isocratic elution (100 % B) for 5 min. The total run time was 55 min at the mobile phase flow rate of 0.4 mL/min.

The SIM function was used for extracts analysis to better visualize the chromatographic separation and to remove signals originating from plant components. The monitored ions were as follows:

313 *m/z* for CBD, Δ8-THC and Δ9-THC;

309 *m/z* for CBN;

327 *m/z* for quinone derivative of CBD (Q-CBD);

345 *m/z* for methoxy derivatives of CBD (CBD-OMe) (i.e. CBD-OMe-1-ad-ba, (*S*)-CBD-OMe-2-ad-c and (*R*)-CBD-OMe-2-ad-c – see discussion below for the explanation of these abbreviations);

349 *m/z* for chlorine derivatives of CBD (CBD-Cl) (i.e. CBD-Cl-1-ad-ba, (*S*)-CBD-Cl-2-ad-c and (*R*)-CBD-Cl-2-ad-c - see discussion below for the explanation of these abbreviations);

355 *m/z* for acyl derivative of CBD (CBD-OAc).

To confirm the identity of the forming CBD derivatives, MS^2 function was applied. The collision energy for each examined compound was chosen individually.

Due to the lack of commercially available standards of some CBD derivatives, i.e. quinone derivative of CBD, methoxy derivatives of CBD, chlorine derivatives of CBD and acyl derivative of CBD, their amounts were estimated by relating their chromatographic responses to the calibration curve for CBD.

2.10. Karl Fischer titration

To estimate the water content in the extracting solvents and plant material, their samples dissolved or suspended in methanol were titrated by Karl Fischer reagent using a V10S Karl Fischer Titrator (Mettler Toledo).

2.11. Formation of high-molecular-weight structures of CBD

As shown in (Daniels et al., 2022), CBD is capable to polymerize and form high-molecular-weight structures. To demonstrate that high-molecular-weight structures can also be formed during high-temperature extraction of hemp, a methanolic CBD solution was subjected to high-pressure extraction. The solution was heated at 225 for 90 min in a tightly closed stainless steel vessel and the dry residue obtained after methanol evaporation was subjected to IR analysis.

2.12. ATR-FTIR

IR spectra of CBD standard and the dry residue obtained after solvent evaporation from thermally treated methanolic CBD solution (see *Formation of high-molecular weight structures of CBD* above) were acquired without any sample pretreatment. The Nicolet™iS50 FTIR spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) with ATR crystal, working in the range from 4000 to 400 cm − 1 was used for this purpose.

3. Results and discussion

The literature and the discussion below indicate that CBD is a molecule that undergoes various transformations and is susceptible to various processes that may occur during its extraction, especially high-temperature extraction, from plant and food products containing CBD. These transformations and processes depend not only on the conditions of the extraction process, but also on the type of extrahent. Their course is also influenced by the content of water and air (specifically oxygen) in the extraction system. The water content in the extraction system depends on its concentration in the extrahent itself and the humidity of the extracted sample, while the air (oxygen) content depends on the extent to which the sample and extrahent fill the high-pressure extraction vessel, air amount dissolved in the extrahent and contained in the sample, and the volume and porosity of the inert material used to fill up the volume of the extraction vessel (if such material is used). For the sake of clarity, the discussion of the influence of extrahent type, water and air content in the extraction vessel on the transformations of CBD during its high-temperature extraction and on a possible formation of high-molecular-weight CBD structures in this process is presented in four thematic blocks:

- the transformations of CBD vs. the extrahent type, when CBD solution or hemp suspension fill the high-pressure extraction vessel by 50 %;
- the transformations of CBD vs. hemp humidity or water content in the extrahent, when CBD solution or hemp suspension fill the high-pressure extraction vessel by 50 %;
- CBD transformation vs. the degree of the extraction vessel filling with the CBD solution or hemp suspension, and
- the presence of high-molecular weight CBD structures in CBD solution during its high temperature treatment.

At the end of the Results and Discussion section, data are presented that prove that CBD, during its high-temperature extraction from CBD containing functional foods, may undergo the same transformations as those observed during hemp extraction.

i Transformations of CBD during its high-temperature extraction with MeOH, DCM, EtOAc or Hex

All results presented in this subsection deal with extraction processes performed using:

- a high-pressure vessel filled in 50 % with CBD solution or hemp suspension;
- dried hemp containing about 9 % of water, and
- solvents with known water concentration (0.08 % in MeOH, 0.02 % in DCM, 0.06 % in Et-OAc and 0.01 % in Hex).

The solvent most often used to isolate CBD from plant material for analytical purposes is methanol. Fig. 1 A shows the GC–MS chromatogram (in TIC) of the methanolic solution of CBD standard, whereas Fig. 1 B presents an exemplary GC–MS chromatogram (in TIC) of the same solution heated at 175 °C for 20 min. This process can be treated as simulated high-temperature extraction of CBD. The chromatogram in Fig. 1 B shows that the process of high-temperature extraction of CBD with methanol leads to the transformation of this cannabinoid and the formation of its 6 derivatives. The GC–MS chromatogram of the

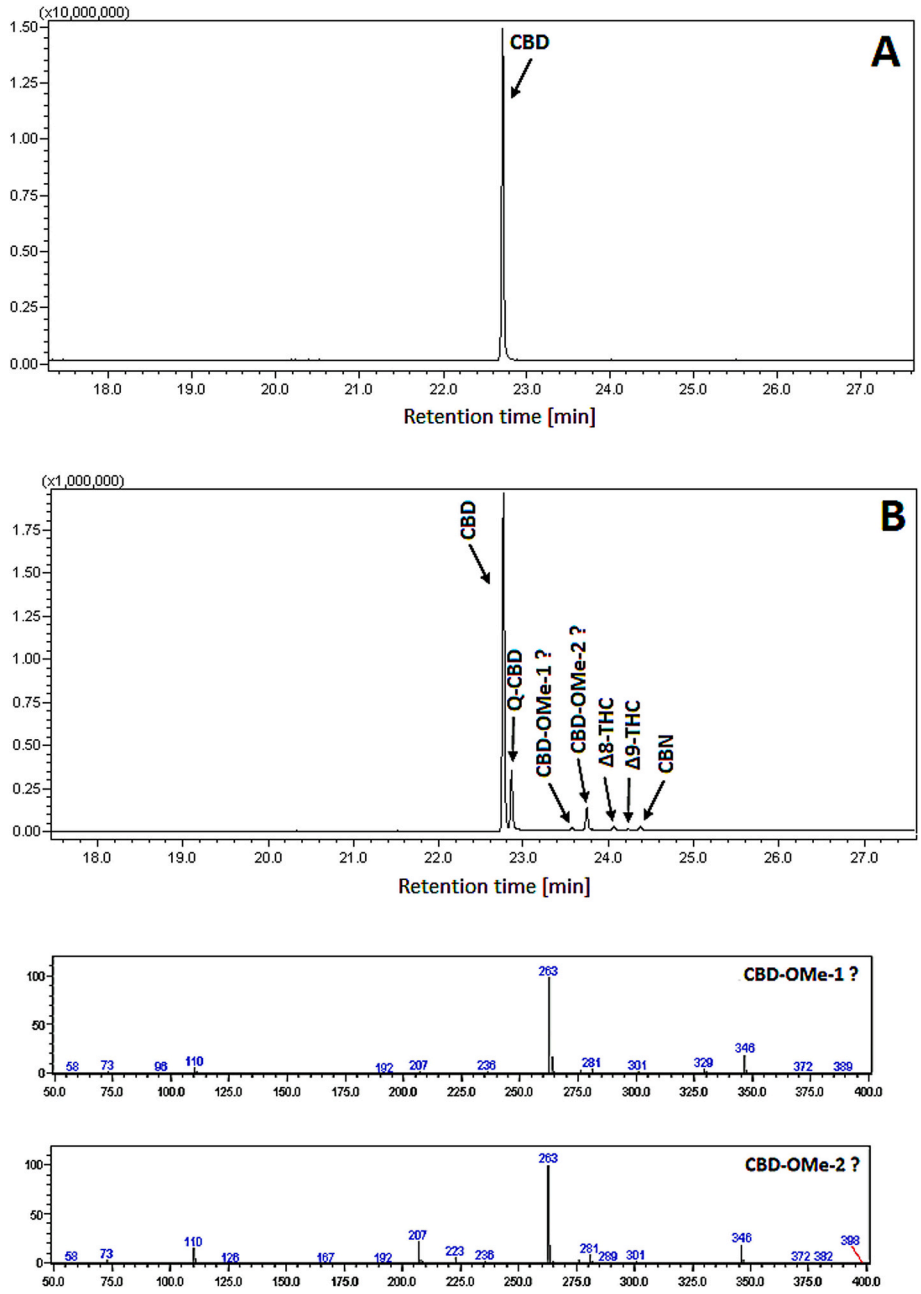

Fig. 1. GC–MS chromatograms (in TIC) of the methanolic solution of CBD standard (A) and of the same solution heated at 175 °C for 20 min (B).

methanolic solution of CBD consists of only one peak (see Fig. 1 A). This fact proves that the CBD derivatives forming in the high-temperature extract of CBD (see Fig. 1 B) are not formed in conditions of the GC injector. Analyzing the chromatogram in Fig. 1 B it is easy to observe that the high-temperature extract contains Δ9-THC, Δ8-THC, and CBN. Their identification results from the comparison of the retention data and GC–MS spectra of these substances with the corresponding data for their standards. Moreover, it is known from the literature that CBD in an acidic environment, and CH_3OH can be considered as such a compound in applied high-temperature extraction conditions, transforms to Δ9-THC, Δ8-THC and CBN, which further confirms the correctness of the identification (Buijs, 2023; Dybowski et al., 2020; Watanabe et al., 2007). The next compound (peak Q-CBD) formed in significant quantities in the process of high-temperature extraction of CBD is its quinone derivative, HU-331 (3-Hydroxy-2-[(1*R*,6*R*)-3-methyl-6-(prop-1-en-2-yl) cyclohex-2-en-1-yl]-5-pentylcyclohexa-2,5-diene-1,4-dione). It was identified by comparing the obtained GC–MS spectrum with its spectrum in the database. The formation of HU-331 in the performed process is very likely due to the presence of oxygen, which is dissolved in methanol and contained in the air filling a part of the extraction vessel used. As for the CBD-OMe-1-ad-? and CBD-OMe-2-ad-? peaks, it is visible that the GC–MS spectra of substances corresponding with them (see spectra below chromatograms in Fig. 1) contain the main ion with $m/z = 263$ and a clearly visible ion with $m/z = 346$. These ions are greater by 32 from the 231 and 314 characteristic for CBD ions. The presence of ions 263 and 346 suggests that the CBD-OMe-1-ad-? and CBD-OMe-2-ad-? peaks correspond to methoxy derivatives of CBD (i.e. methanolic adducts of CBD). This identification is quite probable due to the presence of double bonds in the CBD molecule and the high reactivity of methanol under extreme process conditions (175 °C and high pressure). In order to confirm the identification, and especially the identification of methoxy CBD derivatives, the high-temperature methanolic CBD extracts were analyzed using LC-MS, the method enabling the elimination of secondary reactions such as

– high-temperature reversibility of the addition reaction,
– isomerization of Δ9-THC to Δ8-THC and vice versa and
– dehydrogenation of THC to CBN,

which may affect the quantitative determination of individual CBD derivatives. Moreover, the order of compounds elution in the RP-HPLC system is consistent with their hydrophobicity, which allows the structure to be assigned to individual methoxy derivatives of CBD. LC-MS chromatograms (in SIM) of the methanolic solution of CBD standard and the same solution heated at 175 °C for 20 min are shown in Figs. 2 A and A', respectively. There are 7 peaks in Fig. 2A'. Its analysis, the HRMS and MS^2 data for individual peaks (see Table 1) confirm the correctness of GC–MS identification of CBD derivatives formed during high-temperature heating of the methanolic solution of their precursor. Moreover, it shows the presence of not two but three methoxy CBD derivatives in the solution: CBD-OMe-1-ad-ba, (*S*)-CBD-OMe-2-ad-c, and (*R*)-CBD-OMe-2-ad-c. As the CBD molecule contains two C=C double bonds, one in its branched aliphatic and one in its cyclic structure, the formation of four methoxy derivatives of CBD – which are positional isomers – can be assumed. However, a more thorough analysis indicates that six methoxy derivatives of CBD can be created, four of which are optical isomers (diastereoisomers). Additional designations "ad-ba" and "ad-c" in the short names of methoxy CBD derivatives are used to indicate that they were formed by methanol addition to the branched aliphatic structure of CBD (−ba) or to its cyclic structure (−c). The identification of CBD-OMe-1-ad-ba, (*S*)-CBD-OMe-2-ad-c, and (*R*)-CBD-OMe-2-ad-c peaks is based on the following premises:

– according to Markovnikov's rule, the addition of a nucleophilic substituent to the tertiary carbon atom is more likely than to the secondary carbon atom;
– diastereomeric adducts are formed in similar amounts (see similar sizes of (*S*)-CBD-OMe-2-ad-c and (*R*)-CBD-OMe-2-ad-c peaks);
– greater retention of (*R*)-CBD-OMe-2-ad-c than (*S*)-CBD-OMe-2-ad-c results from its greater hydrophobicity in consequence of the formation of an intramolecular hydrogen bond in the first diastereomer;
– nucleophilic addition to the double bond in a cyclic structure is more probable than in a branched aliphatic structure (a smaller amount of CBD-OMe-1-ad-ba is formed than of each (*S/R*)-CBD-OMe-2-ad-c).

The structures of the identified compounds are shown in section A of Fig. 3.

LC-MS chromatograms (in SIM) of the methanolic hemp macerate and of the high- temperature methanolic extract from the same plant material obtained at 175 °C for 20 min are shown in Fig. 2 B and B′, respectively. A comparison of chromatograms 2 A' and 2B shows that four derivatives of the CBD forming during its high-temperature extraction – Q-CBD, Δ9-THC, Δ8-THC, and CBN – are native components of hemp. As Q-CBD (unlike Δ9-THC, Δ8-THC, and CBN) does not belong to the cannabinoid metabolic pathway of cannabis, its presence in hemp results from secondary exogenous CBD oxidative processes.

The macerate does not contain methoxy CBD derivatives. They are present, however, in the high-temperature hemp extract (see Fig. 2 B′) and are the same methoxy derivatives of CBD as those formed during the high-temperature extraction of CBD standard. There is no information in the current literature about methoxy derivatives of CBD, or about the possibility of their formation during hemp extraction with methanol. Hence, these derivatives, if present in extracts, may be wrongly considered as native components of hemp indicating, in consequence, an unreal metabolic pathway of the plant.

The kinetics of CBD standard transformation and of the formation of its individual derivatives during extraction with methanol at 5 different

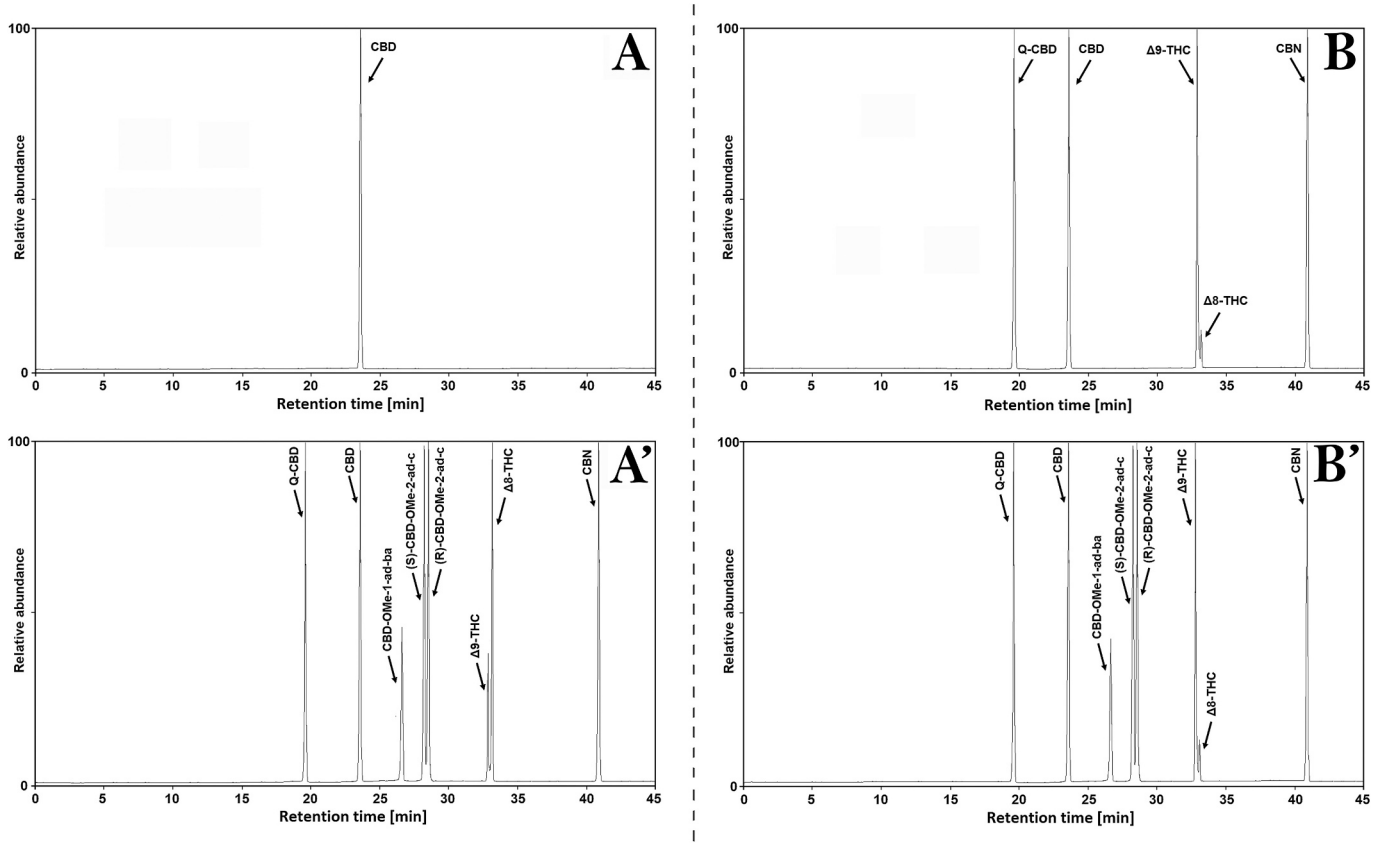

Fig. 2. LC-MS chromatograms (in SIM) of the methanolic solution of CBD standard (A), of the same solution heated at 175 °C for 20 min (A'), of the methanolic hemp macerate (B) and of the high- temperature methanolic extract from the same plant material obtained at 175 °C for 20 min (B′).

Table 1
HRMS and MS^2 data for compounds identified in extracts of CBD standard and hemp, obtained in MeOH, DCM, EtOAc and Hex.

no.	Compound name	Compound shortcut	MS^1	MS^2			HRMS data					Extractant type	Extract type	
			Parent ion	Base peak	Secondary peak		Theoretical mass [M-H] - (Da)	Experimental mass [M-H] - (Da)	Δ mDa	Δ ppm	Elemental composition		CBD standard	Hemp
			m/z	m/z	m/z	Intensity							Analyte presence in extract	
1	2-[(1*R*,6*R*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl]-5-penthylbenzene-1,3-diol	CBD	313.2	257.1	121.2 191.1 296.2	27.5 74.7 17.4	313.21676	313.21665	−0.11	0.35	$C_{21}H_{29}O_2$	MeOH DCM EtOAc Hex	+ + + +	+ + + +
2	(6a*R*,10a*R*)-6,6,9-trimethyl-3- pentyl-6a,7,10,10a-tetrahydro-6H-benzo[*c*] chromen-1-ol	Δ8-THC	313.2	257.1	121.1 191.1 296.2	21.8 68.2 29.7	313.21676	313.21677	0.01	0.03	$C_{21}H_{29}O_2$	MeOH DCM EtOAc Hex	+ + + **nd***	+ + + +
3	(6a*R*,10a*R*)-6,6,9-trimethyl-3-propyl-6a,7,8,10a-tetrahydro-6H-benzo[*c*] chromen-1-ol	Δ9-THC	313.2	257.1	121.1 191.2 296.2	22.4 67.1 31.5	313.21676	313.21685	0.09	0.29	$C_{21}H_{29}O_2$	MeOH DCM EtOAc Hex	+ + + **nd**	+ + + +
4	6,6,9-trimethyl-3- penthyl-6H-benzo [*c*]chromen-1-ol	CBN	309.3	221.2	239.2 291.1	21.5 39.9	309.18546	309.18539	−0.07	0.23	$C_{21}H_{25}O_2$	MeOH DCM EtOAc Hex	+ + **nd** **nd**	+ + + +
5	(1′*R*,6'*R*)-6-hydroxy-3′-methyl-6′-(prop-1-en-2-yl)-4-penthyl-1,1′-bi (cyclohexane)-2′,3,6-triene-2,5-dione	Q-CBD	327.3	299.2	310.2 281.2	57.7 25.7	327.196020	327.196030	0.01	0.03	$C_{21}H_{27}O_3$	MeOH DCM EtOAc Hex	+ + + +	+ + + +
6	2-[(1*R*,6*R*)-6-(2-methoxypropan-2-yl)-3-methylcyclohex-2-en-1-yl]-5-penthylbenzene-1,3-diol	CBD-OMe-1-ad-ba	345.2	313.2	121.2 191.1 257.1 296.2	16.4 47.7 65.8 10.8	345.24297	345.24292	−0.05	0.14	$C_{22}H_{33}O_3$	MeOH DCM EtOAc Hex	+ **nd** **nd** **nd**	+ **nd** **nd** **nd**
7	2-[(1*R*,2*R*,5*S*)-5-methoxy-5-methyl-2-(prop-1-en-2-yl)cyclohexyl]-5-penthylbenzene-1,3-diol	(*S*)-CBD-OMe-2-ad-c	345.2	313.1	121.1 191.1 257.1 296.2	10.2 41.8 59.3 7.5	345.24297	345.24288	−0.09	0.26	$C_{22}H_{33}O_3$	MeOH DCM EtOAc Hex	+ **nd** **nd** **nd**	+ **nd** **nd** **nd**
8	2-[(1*R*,2*R*,5*R*)-methoxy-5-methyl-2-(prop-1-en-2-yl)cyclohexyl]-5-penthylbenzene-1,3-diol	(*R*)-CBD-OMe-2-ad-c	345.2	313.1	121.1 191.2 257.1 296.2	11.3 40.9 59.9 7.1	345.24297	345.24290	−0.07	0.20	$C_{22}H_{33}O_3$	MeOH DCM EtOAc Hex	+ **nd** **nd** **nd**	+ **nd** **nd** **nd**
9	2-[(1*R*,6*R*)-6-(2-chloropropan-2-yl)-3-methylcyclohex-2-en-1-yl]-5-penthylbenzene-1,3-diol	CBD-Cl-1-ad-ba	349.2	313.2	121.2 191.2 257.1 296.2	15.7 48.2 65.1 10.1	349.19343	349.19349	0.06	0.17	$C_{21}H_{30}ClO_2$	MeOH DCM EtOAc Hex	**nd** + **nd** **nd**	**nd** + **nd** **nd**
10	2-[(1*R*,2*R*,5*S*)-5-chloro-5-methyl-2-(prop-1-en-2-yl)cyclohexyl]-5-penthylbenzene-1,3-diol	(*S*)-CBD-Cl-2-ad-c	349.2	313.1	121.1 191.1 257.2 296.2	11.2 42.4 58.8 7.7	349.19343	349.19345	0.02	0.06	$C_{21}H_{30}ClO_2$	MeOH DCM EtOAc Hex	**nd** + **nd** **nd**	**nd** + **nd** **nd**
11	2-[(1*R*,2*R*,5*R*)-5-chloro-5-methyl-2-(prop-1-en-2-yl)cyclohexyl]-5-penthylbenzene-1,3-diol	(*R*)-CBD-Cl-2-ad-c	349.2	313.1	121.2 191.1 257.1 296.1	11.5 41.5 57.8 8.2	349.19343	349.19340	−0.03	0.09	$C_{21}H_{30}ClO_2$	MeOH DCM EtOAc Hex	**nd** + **nd** **nd**	**nd** + **nd** **nd**
12	3-hydroxy-5-penthyl-2-[(1*R*,6*R*)-3-methyl-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl]phenyl acetate	CBD-OAc	355.2	313.2	121.1 191.2 257.1 296.2	15.9 46.4 67.8 11.7	355.22732	355.22731	−0.01	0.03	$C_{23}H_{31}O_3$	MeOH DCM EtOAc Hex	**nd** **nd** + **nd**	**nd** **nd** + **nd**

nd*- not detected.

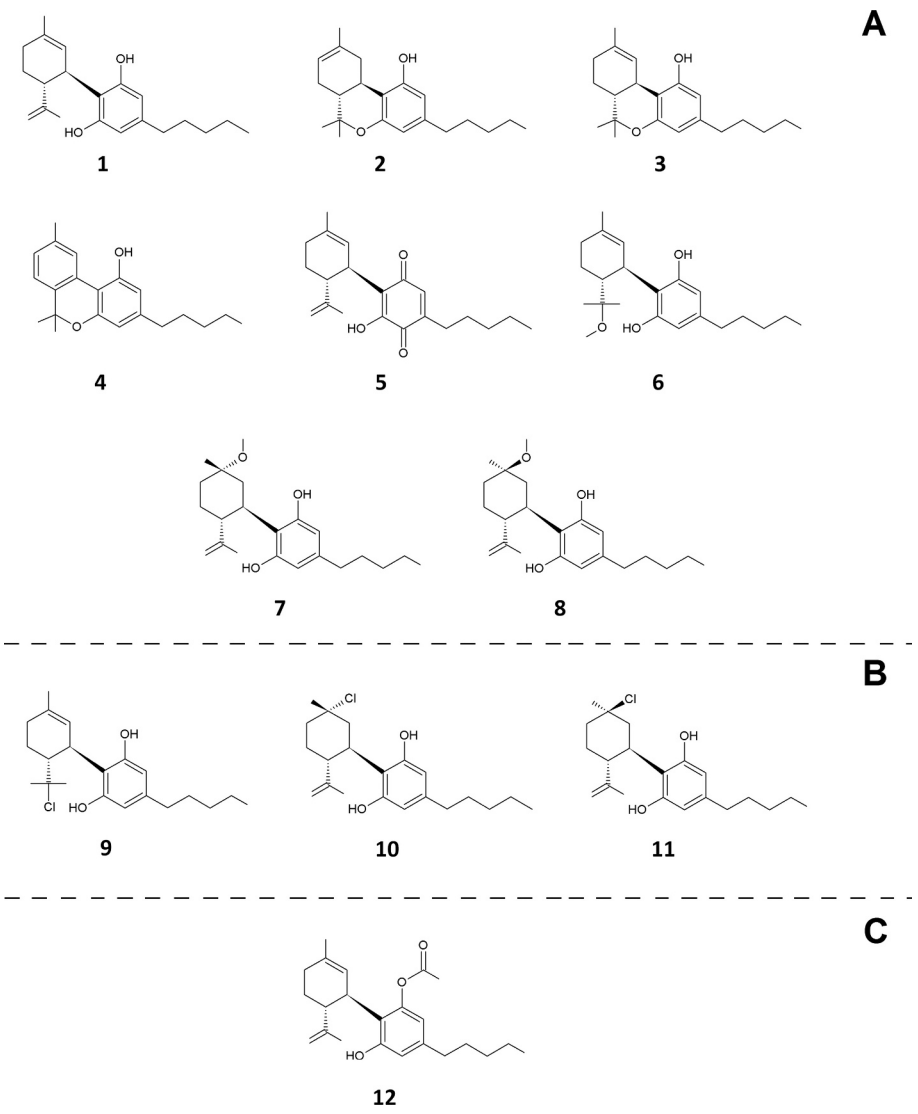

Fig. 3. The structures of CBD derivatives forming during high-temperature extraction of CBD standard or hemp. The full IUPAC names of the presented compounds are given in Table 1.

temperatures (100, 125, 150, 175 or 200 °C) are presented in Fig. 4. The analysis of these relationships indicates that time extension of the process causes:

- an increase of CBD degradation proportional to the process temperature growth;
- an almost monotonic increase of the amount of formed CBN and Q-CBD;
- an increase in the amount of individual methoxyl CBD derivatives with a clear tendency to reach a plateau for their dependencies at higher temperatures, and
- a monotonic increase in the amount of Δ8/Δ9-THC at 125 and 150 °C, and an increase and decrease of their amount at 175 and 200 °C.

The existence of a maximum in the dependencies for Δ8/Δ9-THC is normal for both isomers as intermediate products in the transformation of CBD to CBN.

Fig. 5 shows relationships analogous to Fig. 4, this time for hemp subjected to high-temperature extraction. The initial hemp material contained some amount of Δ9-THC,

Δ8-THC and CBN, which are components of the hemp metabolic pathway, and small amount of Q-CBD, which is not a hemp metabolite but can form in the plant as a result of CBD oxidation (see Fig. 2B). Hence, their relationships should not be treated as the kinetics of their formation, but as the change of their concentration in the extract vs. extraction time increase. This remark applies not only to Fig. 5, but also to all subsequent drawings showing the relationships obtained with hemp material. In order to easily compare the results in Figs. 4 and 5 and to determine the impact of the plant matrix on the formation of individual CBD derivatives, the high-pressure extraction vessel contained enough hemp to reach the same CBD concentration as in the experiments with CBD standard, i.e. ca. 1 mg/mL. Most of the relationships in Fig. 5 are similar to those in Fig. 4. A significant difference is observed for Δ8-THC and Δ9-THC. The concentration of these compounds in the plant extract gets lower with the length of extraction time and the growth of extraction temperature. However, a more detailed comparison of Figs. 4 and 5 shows that in hemp extraction:

- CBD decomposes much slower,
- Q-CBD and CBN are formed in larger quantities, and
- methoxy derivatives of CBD are formed in smaller quantities.

Those differences may be related to the inhibitory (smaller CBD

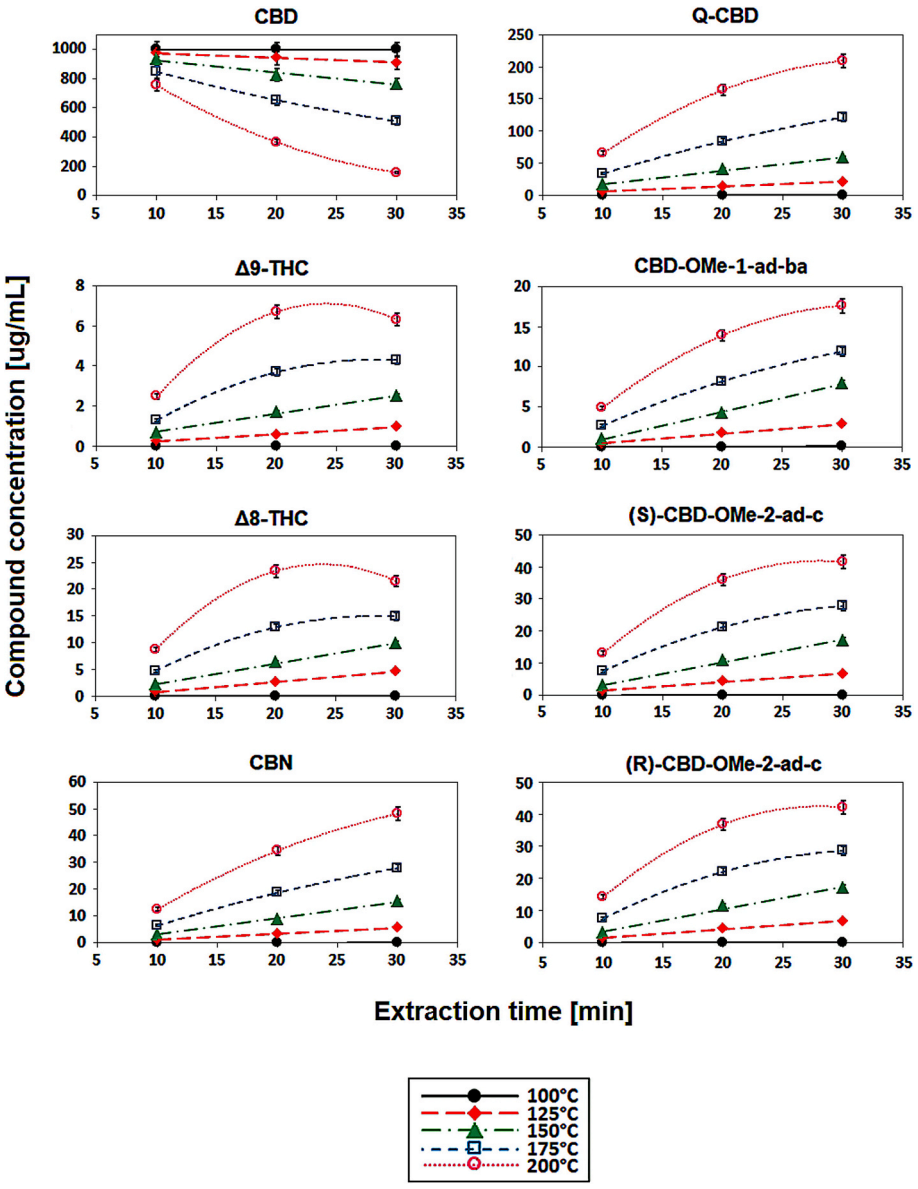

Fig. 4. The kinetics of CBD degradation and of the formation of its individual derivatives (Q-CBD, CBD-OMe-1-ad-ba, (*S*)-CBD-OMe-2-ad-c, (*R*)-CBD-OMe-2-ad-c, Δ9-THC, Δ8-THC and CBN) during CBD standard extraction with methanol at 100, 125, 150, 175 or 200 °C.

decomposition and smaller formation of THC and methoxy derivatives) and catalytic (increased formation of Q-CBD and CBN) effects of the plant matrix. Both opposed effects can be explained by the presence of salts in the plant, which alkalize the extraction environment. The alkaline environment impedes the creation of methoxy derivatives and CBD transformation to Δ9-THC or Δ9-THC isomerization to Δ8-THC. The presence of oxygen in the extraction vessel must not be ignored, either. As some of it is introduced into the vessel together with the plant, the transformation of CBD into Q-CBD and THC into CBN is more effective in the case of hemp than in CBD standard extraction.

Analyzing Figs. 4 and 5, it is worth noting that, the course of the relationships presented in Fig. 5 may be influenced by the efficiency of CBD, Δ8−/Δ9-THC and CBN extraction from hemp, which depends on the temperature and time of the process. However, cannabinoids are secondary hemp metabolites that have a protective effect and are located on the surface of the plant. Their isolation from hemp by high-temperature extraction with a solvent with high solvation power such as MeOH is very effective. Therefore, the impact of the efficiency of CBD, Δ8−/Δ9-THC and CBN extraction from hemp on the course of the relationships presented in Fig. 5 is marginal, if any.

The low boiling point, non-flammability, high density, and medium-polar nature of DCM are the reasons for its frequent use by analysts to extract hydrophobic and medium polar compounds from different matrices, including plant materials. Moreover, DCM is a solvent approved, with certain restrictions, for food processing. GC–MS chromatogram (in TIC) of DCM solution of CBD standard and the chromatogram of this solution after its heating at 175 °C for 20 min are presented in Figs. S1 A and B, respectively. Their comparison proves that the process of high-temperature extraction of CBD with DCM causes its transformation to six derivatives. Four of them are the same as those forming during high-temperature CBD extraction by methanol: Q-CBD, Δ9-THC, Δ8-THC, and CBN. The other two are probably chlorine CBD derivatives (hydrochloric CBD adducts), abbreviated in this work as

CBD-Cl-1-ad-? and CBD-Cl-2-ad-?, formed by the addition of HCl to double bonds in CBD (see Fig. S1 B), especially because DCM is a moderately stable substance that decomposes at elevated temperatures, releasing HCl. This tentative identification of CBD-Cl-1-ad-? and CBD-Cl-2-ad-? results from the analysis of their spectra (see spectra in Fig. S1).

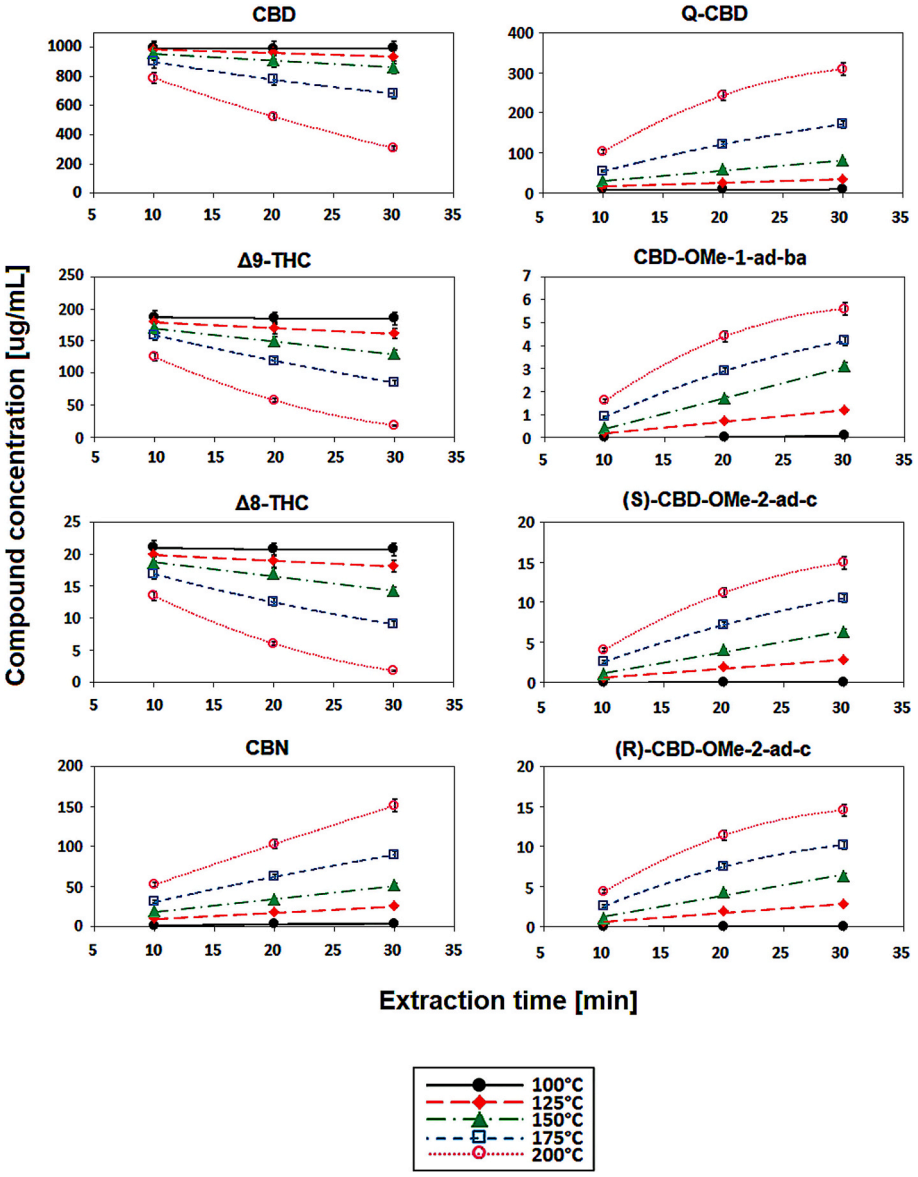

Fig. 5. The influence of the extraction time of hemp with methanol at 100, 125, 150, 175 or 200°C on:
- CBD degradation degree,
- the formation of CBD-OMe-1-ad-ba, (S)-CBD-OMe-2-ad-c, (R)-CBD-OMe-2-ad-c, and
- the concentration change of D8/D9-THC, CBN, and Q-CBD in the final extract.

The GC–MS spectra of the CBD derivatives contain the main ion with $m/z = 267$ and a clearly visible ion with $m/z = 350$. These ions are greater by 36 from the characteristic for CBD ions, which are 231 and 314. Moreover, isotopic ions with m/z 269 and 352 in the spectra of these CBD derivatives also confirm the addition of HCl to CBD.

In order to confirm the formation of chloride CBD derivatives, the high-temperature extract CBD standard and hemp in DCM were analyzed by LC-MS. LC-MS chromatograms (in SIM) of a DCM solution of CBD standard and the same solution heated at 175 °C for 20 min are shown in Figs. S2 A and A', respectively. There are 7 peaks in Fig. S2 A'. Its analysis, the HRMS and MS^2 data for individual peaks (see Table 1) confirm the correctness of GC–MS identification of CBD derivatives formed during high-temperature heating of CBD solution in DCM. Moreover, it shows the presence of not two but three chlorine CBD derivatives in the solution: CBD-Cl-1-ad-ba, (*S*)-CBD-Cl-2-ad-c, and (*R*)-CBD-Cl-2-ad-c. The formation of three hydrochloric CBD adducts is supported by the same premises as those in discussing the creation of three methoxy CBD derivatives. The meaning of the "ad-ba" and "ad-c" abbreviations is also the same. However, one note should be made here. While methoxy CBD derivatives are more hydrophobic than CBD and have a longer retention in the RP-HPLC system, chlorine CBD derivatives are more polar than CBD and therefore stay shorter in the system than CBD. The structures of the identified chlorine CBD derivatives are shown in section B of Fig. 3.

LC-MS chromatograms (in SIM) of hemp macerate in DCM and of the high- temperature plant extract in the same solvent obtained at 175 °C for 20 min are shown in Figs. S2 B and B′, respectively. As can be seen from their comparison, the macerate does not contain chlorine CBD derivatives. They are present, however, in the high-temperature hemp extract (see Fig. S2 B′). The literature lacks information not only about the formation of methoxy CBD derivatives during hemp extraction with methanol. There are also no mentions about the formation of chlorine CBD derivatives during hemp extraction by DCM which, if present in extracts, may be improperly considered as native components of hemp suggesting a wrong metabolic pathway of these plant.

The kinetics dependences relating to CBD degradation and the

formation of its individual derivatives during high-temperature extraction of the CBD standard and hemp with DCM are presented in Figs. S3 and S4, respectively. Their runs and relationships between them are very similar to those observed during the analogous extractions using methanol. A difference in their course concerns only Δ8−/Δ9-THC, which are intermediates in the transformation of CBD to CBN. Comparing the relationships in Figs. S3 and 4 regarding high-temperature extraction of the CBD standard, a higher rate of CBD degradation and

Δ8−/Δ9-THC formation is observed in DCM than in MeOH. The opposite effect is observed for the formation of Q-CDA and CBD adducts, which are form quicker in MeOH than in DCM. Similar conclusions are suggested by the kinetics dependences in Figs. S4 and 5 concerning high-temperature extraction of hemp.

EtOAc is a solvent similar to DCM often used for the extraction of non-polar and medium-polar compounds due to its lower toxicity and lower price in relation to DCM. In addition, EtOAc is less susceptible to the formation of emulsion-like extracts.

GC–MS chromatograms (in TIC) of CBD solution in EtOAc and of the same solution heated at 175 °C for 20 min are presented in Figs. S5 A and B, respectively. Their comparison proves that the process of high-temperature extraction of CBD with EtOAc causes the transformation of the cannabinoid to its four derivatives. Three of them are the same as those forming during high-temperature extraction of CBD by MeOH or DCM: Q-CBD, Δ9-THC, and Δ8-THC. The fourth compound is CBD-OAc, an acyl derivative of CBD formed in the transesterification reaction of CBD with EtOAc. Its identification results from the agreement between its

GC–MS data and the analogous data for the monoacyl derivative of CBD reported in (Typek et al., 2023). The structure of CBD-OAc is presented in section C of Fig. 3. The noticeable absence of CBN in high-temperature CBD extract in EtOAc probably results from the formation of a trace amount of its precursors, Δ9/Δ8-THC, in the extraction process.

LC-MS chromatograms (in SIM) of EtOAc solution of CBD standard and of the same solution heated at 175 °C for 20 min are shown in Figs. S6 A and A', respectively, whereas LC-MS chromatograms (in SIM) of a hemp macerate in EtOAc and of high- temperature plant extract in the same solvent obtained at 175 °C for 20 min, are shown in Fig. S6 B and B′, respectively. The results presented in Fig. S6 A' and B′ confirm the conclusions from Fig. S5 regarding the transformation of CBD in EtOAc. CBN is a native component of hemp and therefore its peak is visible in the chromatogram of the plant extract (see Fig. S6 B′). This cannabinoid is not formed during the transformation of CBD in its EtOAc solution - see chromatograms in Figs. S5 B i S6 A'.

The kinetics dependences relating to CBD degradation and the formation of its individual derivatives during high-temperature extraction of CBD standard and hemp herb with EtOAc are presented in Figs. S7 and S8, respectively. Their runs and relation between them are very similar to those observed during the analogous extractions using MeOH. It should be emphasized, however, that the rate of CBD decomposition and of its derivatives formation in EtOAc is slower than in MeOH and DCM.

Hexane is a classic example of a non-polar solvent often used to extract non-polar compounds. GC–MS chromatogram (in TIC) of hexane solution of CBD standard ~~in~~ and of the same solution heated at 175 °C for 20 min are presented in Figs. S9 A and B, respectively. They show that the high-temperature extracts contain, beside CBD, only a trace amount of

Q-CBD. A similar conclusion results from Fig. S10 showing the LC-MS chromatograms of hexane solution of CBD standard (Fig. S10 A), of the same solution heated at 175 °C for 20 min (Fig. S10 A'), of hexane macerate of hemp (Fig. S10 B), and of high-temperature hexane extract of hemp obtained at 175 °C for 20 min (Fig. S10 B′). The presented chromatograms confirm the presence of Q-CBD in the high-temperature extract of CBD standard.

The kinetics dependences relating to CBD degradation and to the formation of Q-CBD and CBN during high-temperature extraction of CBD standard and hemp with hexane are presented in Figs. S11 and S12, respectively. Their runs indicate that the rate of CBD decomposition and the rate of Q-CBD and CBN formation in hexane is the slowest compared to other solvents used in the experiments.

ii Transformations of CBD during its high-temperature extraction with MeOH, DCM, EtOAc or Hex form hemp of different humidity

All results presented in this subsection deal with extraction processes performed using:

- a high-pressure vessel filled in 50 % with hemp suspension;
- hemp of varying degree of humidity;
- hemp amount sufficient to obtain 1 mg of CBD in 1 mL of hemp suspension, and
- solvents with known water concentration (0.08 % in MeOH, 0.02 % in DCM, 0.06 % in EtOAc, and 0.01 % in Hex) and MeOH with different water concentration.

Although for production and analytical purposes CBD is usually isolated from dry hemp, the degree of its humidity may vary. CBD is a hydrophobic substance that poorly dissolves in water. So, the presence of a different amount of water in the extraction system, resulting from the use of variously dried or even freshly-harvested hemp can not only affect the extracting recovery of CBD but also impact the degree of its transformations during high-temperature extraction. The influence of water content in hemp on CBD degradation and on the concentration change of its individual derivatives in the extract obtained in 20 min extraction at 175 °C using MeOH, DCM, EtOAc or Hex is presented in Fig. S13. The presented relationships demonstrate that an increase in the humidity of plant material causes:

- a clear decrease of CBD degradation in the case of MeOH and DCM, a slight decrease in the case of Et-OAc, and a negligible decrease in the case of Hex (see solid line with full circles in Fig. S13 A'- D′);
- a significant decrease in the formation of:
  CBD-OMe derivatives in the case of MeOH (see dashed line with open diamonds, open squares or open triangles in Fig. S13 A),
  CBD-Cl derivatives in the case of DCM (see dashed line with open diamonds, open squares or open triangles in Fig. S13 B), and
  CBD-OAc derivative in the case of Et-OAc (see dash-dotted line with open diamonds in Fig. S13 C);
- a significant decrease in the formation of Q-CBD derivative in the case of all extrahents (see dotted line with open circles in Fig. S13 A-D);
- a small increase of Δ9-THC concentration, a little bit smaller increase of Δ8-THC concentration (see dotted line with, respectively, full diamonds and full squares Fig. S13 A'-D′), and
- a small decrease of CBN concentration in case of each used extrahent type (see dash-dotted line with full triangles in Fig. S13 A'-D′).

As indicated above, under high-temperature extraction conditions CBD standard transforms, among others, to Δ8-THC, Δ9-THC, and CBN, which are native hemp components. The last two observations above result from the influence of hemp humidity on three processes: (1) the transformation of CBD into these cannabinoids, (2) the efficiency of their extraction from plant material, and (3) the degradation of Δ9−/Δ8-THC towards the formation of CBN. A reminder supplementing the above conclusions is that the transformation of CBD into Δ9−/Δ8-THC, CBN, Q-CBD, CBD-OMe, and CBD-Cl (i.e. into all examined CBD derivatives, except CBD-OAc) requires the activation of a double bond in its molecule and the formation of a carbocation. The water present in the extraction system, interacting with the carbocation, hinders its further transformations to the mentioned CBD derivatives. The water present in the extraction system also affects the formation of the ester bond in CBD-OAc and causes its hydrolysis. Therefore, the observed decrease of CBD transformations into all CBD derivatives resulting from the increase of

hemp humidity is understandable. To confirm this, the transformation of the CBD standard was tested during its high-temperature extraction with MeOH containing water in the range of 0.25–5.0 %. The maximum water concentration in methanol was greater to that in the extraction system when the most humid hemp was extracted. As results from Fig. S14 presenting the results of these experiments, a small amount of water drastically reduces transformation of CBD into its derivatives, thus confirming protective activity of water towards this cannabinoid. This is a very important finding both for the accuracy of CBD analysis and for its isolation from hemp for production/commercial purposes.

iii The effect of changing air content in the extraction vessel on the transformations of CBD during its high-temperature extraction from hemp by MeOH, DCM, EtOAc or Hex

All results presented in this subsection deal with extraction processes performed using:

- dried hemp containing about 9 % of water;
- hemp amount sufficient to obtain 1 mg of CBD in 1 mL of hemp suspension, and
- solvents with known water concentration (0.08 % in MeOH, 0.02 % in DCM, 0.06 % in EtOAc, and 0.01 % in Hex).

The results described in previous subsections were obtained using a high-pressure extraction vessel filled to 50 % of its volume with a solution of CBD standard or hemp suspension. The remaining volume of the vessel was air saturated with the vapor of the extracting liquid. The presence of oxygen has a significant impact on the CBD transformation process, causing, for instance, CBD transformation to Q-CBD or supporting the formation of CBN from THC. Fig. S15 presents the influence of air content in the extraction vessel on:

- CBD degradation,
- the formation of, methoxy, chlorine, and acyl derivatives of CBD, and
- the concentration change of Δ8/Δ9-THC, CBN and Q-CBD in the final extract

which occur during 20 min hemp extraction at 175 °C using MeOH, DCM, EtOAc or Hex. The plots showing the increase of Q-CBD and CBN concentration in the final extract resulting from the air volume increase in the extraction vessel confirm the importance of oxygen in the transformation process of CBD and THC to these two derivatives. Therefore, the decrease in CBD and THC, when the volume of air in the extraction vessel increases, is obvious. It is worth noting that the strongest impact of the air volume increase in the extraction vessel on the concentration change of CBD, THC, Q-CBD, and CBN in the hemp extract is observed in the case of DCM and MeOH, while it is the smallest in the case of EtOAc and Hex (see part A in Fig. S15). As to methoxy, chlorine, and acyl derivatives of CBD, their formation decreases with the air volume increase in the extraction vessel (see part B in Fig. S15). As the formation of CBD adducts or acyl derivative of CBD is an ionic reaction, not a radical one, the decrease in the amount of these derivatives with the air volume increase in the extraction vessel most likely results from the decrease in the amount of their precursor, CBD, in the extraction system due to its consumption in the transformations described above.

Considering the influence of the air amount in the extraction vessel on possible CBD transformation during its high-temperature extraction, a small amount of air dissolved in the extracting liquid or contained in hemp must not be forgotten. Difficulties in the assessment of its amount did not allow us to determine its additional effect on CBD transformation in the extraction process.

iv Formation of high molecular weight derivatives of CBD during its high-temperature extraction with MeOH

CBD is a complex molecule containing two double bonds, one in its cyclic and one in its branched aliphatic part, which makes CBD capable of polymerization, as reported in (Daniels et al., 2022). If so, can the high-molecular-weight structures of CBD be formed during the high-temperature extraction of hemp? This is a valid question as the extraction system contains small amounts of oxygen that reveals its radical properties at high temperature, promoting radical polymerization of unsaturated compounds. Consequently the formation of sparingly soluble macromolecular forms of CBD during its high-temperature extraction may affect the reliability of ~~the~~ its quantitative estimation. Since it is difficult to determine the presence of macromolecular forms of CBD in plant sediment after hemp extraction, the CBD standard was used in the research.

Fig. S16 shows two spectra, one of CBD standard and one of the dry residue obtained after evaporation of solvent from the high-temperature (225 °C, 90 min) methanolic extract of CBD standard. The comparison of the spectra clearly shows that the aromatic structures of CBD and its formations contained in the dry residue are very similar. However, in the spectrum of the dry residue there is no band with wave number 3407 cm^{-1} which corresponds to the phenolic -OH group, and no band with wave number 3079 cm^{-1} corresponding to the double bond; yet there is a band 1136 cm^{-1} corresponding to the ether group. The difficult solubility of the dry residue and its spectrum indicates that it contains formations of higher molecular weights than CBD. In these formations ether bonds are present. Most probably, under high-temperature extraction conditions, the CBD molecules combine with each other by the reaction of the hydroxyl group belonging to the phenolic moiety of one molecule with a double bond in the aliphatic or cyclic part of the other molecule (in reaction addition). Taking into account the CBD molecule steric hindrance, the attack of -OH group on the double bond in the branched aliphatic chain is most likely.

Transformation of CBD during its high-temperature extraction from CBD containing functional foods.

All results presented below deal with extraction processes performed using:

- methanol containing 0.08 % of water;
- a high-pressure vessel filled in 50 % with CBD containing foodstuff and methanol.

Great interest in the bioactive properties of CBD has resulted in the appearance on the market not only of medicines and dietary supplements with this cannabinoid, but also of food products classified as functional food. The diagrams in Fig. 6 compare the concentration of CBD and its derivatives in methanolic extracts from CBD containing commercially available food products, which were obtained in the low- (25 °C) and high-temperature (175 °C) extraction process. 25 and 175 °C were chosen for comparison because: (1) CBD derivatives are not formed at low temperatures, (2) a linear increase in the concentration of CBD derivatives is observed in the range of 100–175 °C with increasing extraction time, (3) decomposition of CBD derivatives is observed above 175 °C (see data in Fig. 4). In order to easily compare the results in Fig. 6 and to determine the impact of the food matrix on the formation of individual CBD derivatives, the high-pressure extraction vessel contained such amount of examined foodstuff to reach CBD amount equal 5 mg in it. Hence, the initial CBD concentration related to the volume of extrahent contained in extraction vessel was ca. 1 mg/mL (see Experimental, section: *Low- and high-temperature extraction of CBD containing foodstuffs*). As can be seen, high-temperature extraction of functional foods leads to the formation of the same CBD derivatives as those forming in high-temperature extraction of CBD standard with methanol (compare Fig. 6 with Fig. 2 and/or Fig. 4). A comparison of individual diagrams indicates, however, that the transformation degree of CBD into its individual derivatives depends on the food product composition. While in the case of chips, chocolate and coffee, the transformation of CBD to its derivatives is similar (see similar quantitative relations between individual CBD derivatives in the extracts from these foods), in the case of other foodstuffs containing CBD, cookies, jelly candy, lollypop and hemp-tea, it is different. The higher concentration of Q-CBD and CBN in cookie extract than in chips, chocolate and coffee extracts, is most likely

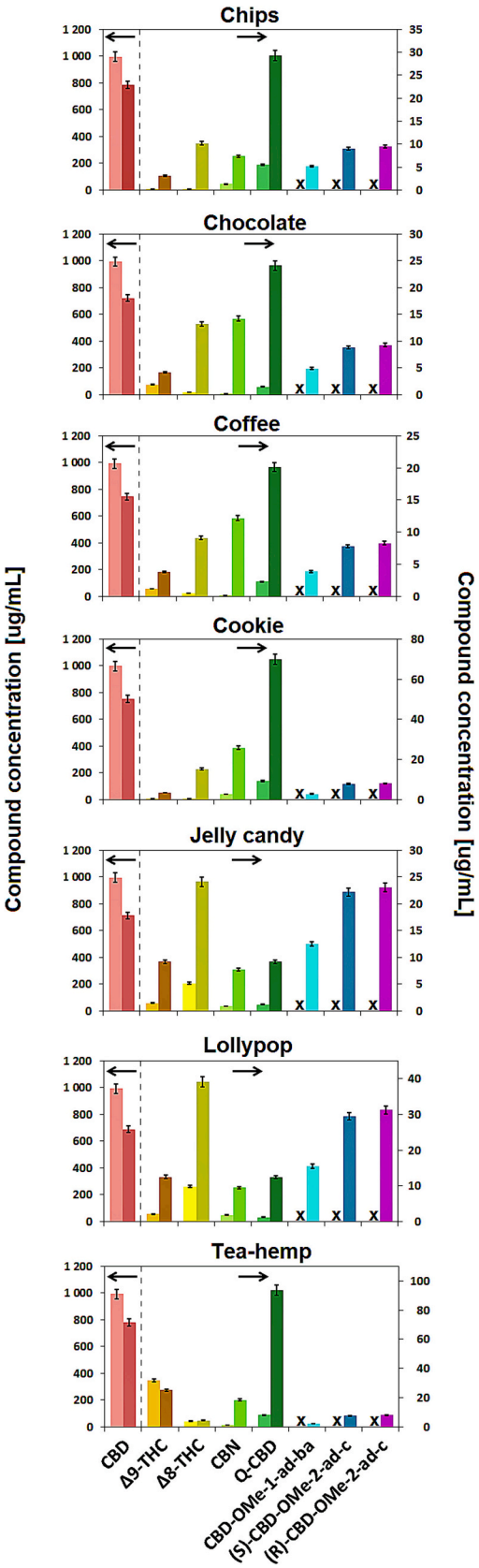

Fig. 6. The concentrations of CBD and its derivatives in methanolic extracts from CBD containing commercially available food products, which were obtained in the low- and high-temperature extraction process (left and right bars, respectively).

the result of the presence of alkaline $NaHCO_3$ in cookie matrix, which is used as a leavening agent. In turn, low concentrations of Q-CBD and CBN and high concentrations of THC isomers and methoxyl CBD derivatives in jelly candy and lollypop extracts is associated with the acidic nature of these foodstuffs due to the presence of citric and ascorbic acids in them. The quantitative relations between CBD derivatives in hemp-tea extract are similar to those characteristic of hemp extract. An exemplary LC-MS chromatogram (in SIM) of a low- and high-temperature methanol extract of jelly candy is shown in fig. S17 (see supplementary materials).

4. Conclusions

During its high-temperature extraction, CBD is transformed into derivatives, the number and quantity of which depend mainly on the type of extracting liquid and water and air (oxygen) content in the extraction system. The largest number of CBD transformation products are formed during extraction with MeOH and DCM. In the case of these two solvents, CBD extract, apart from Δ8-THC, Δ9-THC, CBN, and Q-CBD, also contains three methoxy and three chlorine derivatives of CBD. These CBD derivatives have not yet been reported in the literature. The number of CBD derivatives formed during CBD extraction with EtOAc is slightly smaller. In this case, in addition to 8-THC, 9-THC, CBN and Q-CBD, an acyl derivative of CBD, CBD-OAc, is formed. The smallest number of CBD derivatives is observed in Hex. Of the above-mentioned derivatives, only Q-CBD can be found in the high-temperature hexane extract of CBD standard. The formation of Q-CBD, the quinone derivative of CBD, during its high-temperature extraction is the result of air (oxygen) presence in the extraction system. The more air there is in the system, the greater the conversion degree of CBD into Q-CBD. For each tested extrahent, the humidity of the extracted hemp has a very significant impact on the degree of CBD transformation into each of its derivatives: the higher it is, the lower the transformation degree. A small amount of water drastically reduces transformation of CBD into its derivatives, thus confirming protective activity of water towards this cannabinoid. As results from the performed experiments, in addition to low-molecular-weight CBD transformation products, high-molecular-weight formations may also be formed during its high-temperature extraction, which probably result from the reaction of mutual CBD molecules addition.

The research with the use of chips, chocolate, coffee, cookies, jelly candy, lollipops and hemp tea has shown that analogous CBD transformation products may be formed during high-temperature extraction of functional foods containing this cannabinoid. This last statement is very important in view of the increasing popularity of food products and dietary supplements containing CBD, which should be subject to analytical control, as noted in Introduction.

An additional conclusion resulting from the research is that limiting the formation of the number of derivatives formed in the process of high-temperature extraction of hemp and functional foods/dietary supplements containing CBD can be achieved by using hexane or methanol containing up to 5 % water as the extraction medium.

The results presented here are valid both for the analysis of CBD-containing edibles and for the analysis of hemp itself and its metabolites. On the one hand, they indicate what errors can be made in quantitative assessment of individual native cannabinoids when high-temperature solvent extraction is used as a sample preparation method for analysis of hemp and foods containing CBD. On the other hand, they prove that during such extraction previously unknown CBD derivatives may be formed, which are not necessarily metabolites of the plant.

CRediT authorship contribution statement

Rafal Typek: Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Michal P. Dybowski:** Writing – original draft, Visualization, Methodology, Investigation,

Formal analysis, Conceptualization. **Andrzej L. Dawidowicz:** Writing – original draft, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

- CBD degradation degree,
- the formation of CBD-OMe-1-ad-ba, (*S*)-CBD-OMe-2-ad-c, (*R*)-CBD-OMe-2-ad-c, and
- the concentration change of Δ8/Δ9-THC, CBN, and Q-CBD in the final extract.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.143447.

Data availability

All data generated or analyzed during the study are included in the present article.

References

Aili, Q., Cui, D., Yang, L., Zhige, W., Yongping, W., Minfen, Y., Dongbin, L., Xiao, R., & Qiang, W. (2024). Composing functional food from agro-forest wastes: Selectively extracting bioactive compounds using supercritical fluid extraction. *Food Chemistry, 455*, Article 139848. https://doi.org/10.1016/j.foodchem.2024.139848

Amin, M. R., & Ali, D. W. (2019). Pharmacology of medical Cannabis. *Advances in Experimental Medicine and Biology, 1162*, 151–165. https://doi.org/10.1007/978-3-030-21737-2_8/COVER

Bartončíková, M., Lapčíková, B., Lapčík, L., & Valenta, T. (2023). Hemp-derived CBD used in food and food supplements. *Molecules, 28*, 8047. https://doi.org/10.3390/molecules28248047

Bitwell, C., Indra, S. S., Luke, C., & Kakoma, M. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African, 19*, Article e01585. https://doi.org/10.1016/j.sciaf.2023.e01585

Buijs, W. (2023). The scientific controversy on the conversion of CBD into THC in the human stomach: Molecular modelling and experimental results compared. *Forensic Chemistry, 32*, Article 100467. https://doi.org/10.1016/j.forc.2023.100467

Caffarel, M. M., Andradas, C., Pérez-Gómez, E., Guzmán, M., & Sánchez, C. (2012). Cannabinoids: A new hope for breast cancer therapy? *Cancer Treatment Reviews, 38*, 911–918. https://doi.org/10.1016/j.ctrv.2012.06.005

Caprioglio, D., Mattoteia, D., Taglialatela-Scafati, O., Muñoz, E., & Appendino, G. (2021). Cannabinoquinones: Synthesis and biological profile. *Biomolecules, 11*, 991. https://doi.org/10.3390/biom11070991

Christinat, N., Savoy, M-C., & Mottier, P. (2020). Development, validation and application of a LC-MS/MS method for quantification of 15 cannabinoids in food. Food Chemistry, 318, 126469. doi:https://doi.org/10.1016/j.foodchem.2020.126469.

Daniels, R., Morato, E. O., Yassin, O. A., Mao, J., Mutlu, Z., Jain, M., … Sotzing, G. A. (2022). Poly(cannabinoid)s: Hemp-derived biocompatible thermoplastic polyesters with inherent antioxidant properties. *ACS Applied Materials & Interfaces, 14*, 42804–42811. https://doi.org/10.1021/acsami.2c05556

Dawidowicz, A. L., Dybowski, M. P., Typek, R., Rombel, M., & Holowinski, P. (2023). Unexpected formation of dichloroacetic and trichloroacetic artefacts in gas chromatograph injector during Cannabidiol analysis. *Journal of Pharmaceutical and Biomedical Analysis, 230*, Article 115388. https://doi.org/10.1016/j.jpba.2023.115388

De Aquino, J. P., Bahji, A., & D'Souza, D. C. (2022). Letter to the editor: cannabis as a solution to the opioid crisis: Is the cart before the horse again? *Cannabis and Cannabinoid Research, 7*, 898–900.

Devinsky, O., Cilio, M. R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., Katz, R., Di Marzo, V., Jutras-Aswad, D., Notcutt, W. G., Martinez-Orgado, J., Robson, P. J., Rohrback, B. G., Thiele, E., Whalley, B., & Friedman, D. (2014). Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia, 55*, 791–802. https://doi.org/10.1111/epi.12631

Dybowski, M. P., Dawidowicz, A. L., Typek, R., & Rombel, M. (2020). Conversion of cannabidiol (CBD) to Δ9-tetrahydrocannabinol (Δ9-THC) during protein precipitations prior to plasma samples analysis by chromatography – Troubles with reliable CBD quantitation when acidic precipitation agents are applied. *Talanta, 220*, Article 121390. https://doi.org/10.1016/j.talanta.2020.121390

Holowinski, P., Typek, R., Dawidowicz, A. L., Rombel, M., & Dybowski, M. P. (2022). Formation of trifluoroacetic artefacts in gas chromatograph injector during Cannabidiol analysis. *Journal of Chromatography A, 1671*, Article 463020. https://doi.org/10.1016/j.chroma.2022.463020

Hsu, Y. H., Fang, M. C., Huang, S. C., Kao, Y. M., Tseng, S. H., & Wang, D. Y. (2021). Determination of cannabinoids in hemp oil based cosmetic products by LC-tandem MS. *Journal of Food and Drug Analysis, 29*, 502–509. https://doi.org/10.38212/2224-6614.3370

Kogan, N. M., Peters, M., & Mechoula, R. (2021). Cannabinoid Quinones—A review and novel observations. *Molecules, 26*, 1761. https://doi.org/10.3390/molecules26061761

Lazarjani, M. P., Young, O., Kebede, L., & Seyfoddin, A. (2021). Processing and extraction methods of medicinal cannabis: A narrative review. *Journal of Cannabis Research, 3*, 32. https://doi.org/10.1186/s42238-021-00087-9

Lo Faro, A. F., Venanzi, B., Pilli, G., Ripani, U., Basile, G., Pichini, S., & Busardò, F. P. (2022). Ultra-high-performance liquid chromatography-tandem mass spectrometry assay for quantifying THC, CBD and their metabolites in hair. Application to patients treated with medical cannabis. *Journal of Pharmaceutical and Biomedical Analysis, 217*, Article 114841. https://doi.org/10.1016/J.JPBA.2022.114841

Marchioni, C., Vieira, T. M., Miller Crotti, A. E., Crippa, J. A., & Costa Queiroz, M. E. (2020). In-tube solid-phase microextraction with a dummy molecularly imprinted monolithic capillary coupled to ultra-performance liquid chromatography-tandem mass spectrometry to determine cannabinoids in plasma samples. *Analytica Chimica Acta, 1099*, 145–154. https://doi.org/10.1016/j.aca.2019.11.017

Massi, P., Solinas, M., Cinquina, V., & Parolaro, D. (2013). Cannabidiol as potential anticancer drug. *British Journal of Clinical Pharmacology, 75*, 303–312. https://doi.org/10.1111/J.1365-2125.2012.04298.X

McGuire, Ph., Robson, Ph., Cubala,W.J., Vasile, D., Morrison, P.D., Barron, R., Taylor, A., & Wright, S. (2018). Cannabidiol (CBD) as an adjunctive therapy in schizophrenia: A multicenter randomized controlled trial. American Journal of Psychiatry, 175, 225–231. doi:https://doi.org/10.1176/appi.ajp.2017.17030325.

McMurry, J. (2007). *Organic chemistry* (7th ed.). Belmont: Brooks/ColeThomson.

Mechoulam, R., & Hanuš, L. (2002). Cannabidiol: An overview of some chemical and pharmacological aspects. Part I: Chemical aspects. *Chemistry and Physics of Lipids, 121*, 35–43. https://doi.org/10.1016/S0009-3084(02)00144-5

Moret, S., Scolaro, M., Barp, L., Purcaro, G., Sander, M., & Conte, L. S. (2014). Optimisation of pressurised liquid extraction (PLE) for rapid and efficient extraction of superficial and total mineral oil contamination from dry foods. *Food Chemistry, 157*, 470–475. https://doi.org/10.1016/j.foodchem.2014.02.071

Myers, C., Herrington, J. S., Hamrah, P., & Anderson, K. (2021). Accelerated solvent extraction of terpenes in Cannabis coupled with various injection techniques for GC-MS analysis. *Frontiers in Chemistry, 9*, Article 619770. https://doi.org/10.3389/fchem.2021.619770

Palmieri, B., Laurino, C., & Vadalà, M. (2017). Short-term efficacy of CBD-enriched hemp oil in girls with Dysautonomic syndrome after human papillomavirus vaccination. *The Israel Medical Association Journal, 19*, 79–84. https://europepmc.org/article/med/28457055.

Pertwee, R. G. (2005). Cannabidiol as a potential medicine, in: Cannabinoids as Ther. *Birkhäuser-Verlag*, 47–65. https://doi.org/10.1007/3-7643-7358-x_3

Pisciottano, I. D. M., Guadagnuolo, G., Soprano, V., Esposito, M., & Gallo, P. (2021). A survey of Δ9-THC and relevant cannabinoids in products from the Italian market: A study by LC–MS/MS of food, beverages and feed. *Food Chemistry, 346*, Article 128898. https://doi.org/10.1016/j.foodchem.2020.128898

Rock, E. M., Bolognini, D., Limebeer, C. L., Cascio, M. G., Anavi-Goffer, S., Fletcher, P. J., Mechoulam, R., Pertwee, R. G., & Parker, L. A. (2012). Cannabidiol, a nonpsychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT 1A somatodendritic autoreceptors in the dorsal raphe nucleus. *British Journal of Pharmacology, 165*, 2620–2634. https://doi.org/10.1111/j.1476-5381.2011.01621.x

Trac, J., Keck, J. M., & Deweese, J. E. (2021). Cannabidiol oxidation product HU-331 is a potential anticancer cannabinoid-quinone: A narrative review. *Journal of Cannabis Research, 3*, 11. https://doi.org/10.1186/s42238-021-00067-z

Typek, R., Holowinski, P., Dawidowicz, A. L., Dybowski, M. P., & Rombel, M. (2023). Chromatographic analysis of CBD and THC after their acylation with blockade of compound transformation. *Talanta, 251*, Article 123777. https://doi.org/10.1016/j.talanta.2022.123777

Vlad, R. A., Farczádi, L., Toma, C. M., Imre, S., Antonoaea, P., Rédai, E. M., Muntean, D. L., & Ciurba, A. (2021). Cannabidiol content evaluation in commercial dietary supplements and stability in oil vehicle. *Studia Universitatis Babes-Bolyai, Chemia, 2*, 299–308.

Watanabe, K., Itokawa, Y., Yamaori, S., Funahashi, T., Kimura, T., Kaji, T., Usami, N., & Yamamoto, I. (2007). Conversion of cannabidiol to Δ9-tetrahydrocannabinol and related cannabinoids in artificial gastric juice, and their pharmacological effects in mice. *Forensic Toxicology, 25*, 16–21. https://doi.org/10.1007/s11419-007-0021-y