PAPER

Combined ionic liquid and supercritical carbon dioxide based dynamic extraction of six cannabinoids from *Cannabis sativa* L.†

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The potential of supercritical CO_2 and ionic liquids (ILs) as alternatives to traditional extraction of natural compounds from plant material is of increasing importance. Both techniques offer several advantages over conventional extraction methods. These two alternatives have been separately employed on numerous ocassions, however, until now, they have never been combined for the extraction of secondary metabolites from natural sources, despite properties that complement each other perfectly. Herein, we present the first application of an IL-based dynamic supercritical CO_2 extraction of six cannabinoids (CBD, CBDA, Δ^9-THC, THCA, CBG and CBGA) from industrial hemp (*Cannabis sativa* L.). Various process parameters were optimized, *i.e.*, IL-based pre-treatment time and pre-treatment temperature, as well as pressure and temperature during supercritical fluid extraction. In addition, the impact of different ILs on cannabinoid extraction yield was evaluated, namely, 1-ethyl-3-methylimidazolium acetate, choline acetate and 1-ethyl-3-methylimidazolium dimethylphosphate. This novel technique exhibits a synergistic effect that allows the solvent-free acquisition of cannabinoids from industrial hemp, avoiding further processing steps and the additional use of resources. The newly developed IL-based supercritical CO_2 extraction results in high yields of the investigated cannabinoids, thus, demonstrating an effective and reliable alternative to established extraction methods. Ultimately, the ILs can be recycled to reduce costs and to improve the sustainability of the developed extraction process.

Introduction

Cannabis sativa L. is an annual herbaceous blossoming plant that has been used throughout history in the textile industry, for recreational purposes and in medical applications. It is regarded as one of the oldest cultivated plants, and one of the most essential crops for the progress of humankind. Although native to Eastern Asia, its extensive applications led to its global spread.[1]

The medicinal properties of *Cannabis sativa* L. can be attributed to the many bioactive compounds present in the plant, such as terpenes, polyphenols, phytosterols, tocopherols, fatty acids, and, specifically, cannabinoids, which are terpenophenolic secondary metabolites.[2,3] It is important to mention that
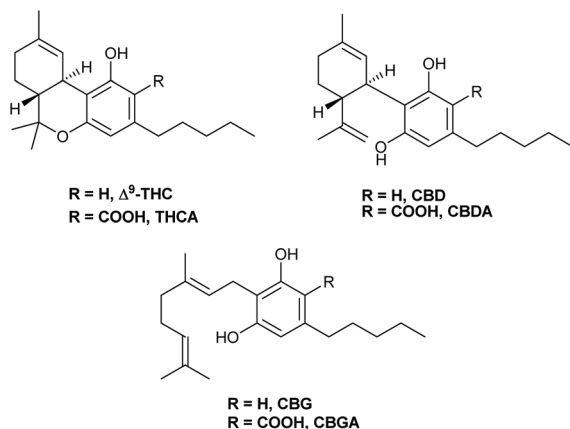
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cannabinoids are not equally distributed in the plant. They are mainly found in the trichomes and in smaller to negligible amounts in the seeds, while roots contain none.[4]

Presently, over 100 cannabinoids have been identified.[5] They are primarily encountered in their carboxylated form in the plant which constitutes a structure of 22 carbon atoms. So far, cannabinoids have been categorized into 11 subclasses: (1) (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), (2) (−)-Δ^8-tetrahydrocannabinol (Δ^8-THC), (3) cannabidiol (CBD), (4) cannabigerol (CBG), (5) cannabichromene (CBC), (6) cannabinol (CBN), (7) cannabinodiol (CBND), (8) cannabicyclol (CBL), (9) cannabielsoin (CBE), (10) cannabitriol (CBT) and (11) miscellaneous. The structures of cannabinoids from hemp investigated in this study are depicted in Fig. 1.[6]

In terms of the biosynthesis of cannabinoids, CBGA is the main precursor for THCA and CBDA.[7] However, under high temperatures, both acids are prone to degrade into their respective decarboxylated analogues, Δ^9-THC and CBD.[8]

Δ^9-THC and CBD are the most abundant cannabinoids present in cannabis plants. Δ^9-THC is well-known as a psychoactive compound, which influences the central nervous and cardiovascular systems. Contrarily, CBD is non-psychoac-

Fig. 1 Structures of the investigated cannabinoids in this study: Δ^9-THC, THCA, CBD, CBDA, CBG and CBGA.

tive, but is regarded as a compound of enormous medical interest, as it has demonstrated numerous health benefits. It has been reported to have anti-inflammatory, antiepileptic and anticonvulsive properties, among many others.[9–11] Excellent medicinal potential have been attributed to cannabinoids; thus, significant effort has been made in the past decades towards the research of the functions and mechanisms of cannabis-derived secondary metabolites in the human body.

Due to the growing medicinal interest in cannabinoids over the years, scientists have undertaken efforts in the development of extraction methods for these valuable bioactive compounds. Traditionally, Δ^9-THC and other cannabinoids have been isolated by solvent-based extractions, with hydrocarbons and alcohols delivering the highest yields.[12,13] Soxhlet extraction (SE) is also a commonly used technique,[14,15] which is characterized by shortcomings, namely, long extraction times and high temperature that may promote thermal degradation of the target compounds.[16]

Other advanced extraction techniques, such as microwave-assisted extraction (MAE) allow higher yields, shorter extraction times, less solvent and reduced energy consumption.[14,17] Nevertheless, uneven heating and/or overheating may cause thermal degradation, and thus negatively impact the extraction efficiency.[18] Alternatively, the use of ultrasound-assisted extraction (UAE) achieves high yields in short times;[19] however, the distribution of ultrasound energy lacks uniformity and over time the power decreases, which can lead to inefficient use of the ultrasound-generated energy.[20]

Supercritical fluid extraction (SFE) is an innovative separation technique, which has thus far been employed for extractions of valuable constituents from over 300 plant species.[21] Carbon dioxide is a widespread choice for SFEs due to its several advantageous properties, such as low reactivity, non-toxicity, non-flammability, affordability, availability, and recyclability. Additionally, its selectivity can be adjusted by modification of pressure and temperature, while product fractionation and recovery with high purity is feasible. Nevertheless, due to its low polarity, addition of small quantities of organic

solvents (co-solvents or modifiers) is necessary to access more polar compounds, thereby expanding its extraction range.[22] The selection of an appropriate co-solvent is key for achieving optimum solubility of the bioactive compounds present in the plant.[23] Supercritical carbon dioxide has previously been used to assess the solubility of individual cannabinoids, for example, Δ^9-THC,[24] CBD[25] and CBG.[25] Moreover, several extractions of cannabinoids from different parts of the cannabis plant, for instance, leaves, trimmings, buds, flowers and threshing residues, have been performed using ethanol as a co-solvent.[26–29]

Within the past years, ionic liquids have also emerged as alternative reaction media for the extraction of biomass that is regarded as a source of natural medicinally relevant complex compounds. Many different properties are attributed to ionic liquids, such as exceptional dissolution properties, high thermal stability and broad liquid range, to name a few. Furthermore, ILs display high tuneability, as the combination of different cations and anions leads to hydrophilicity or hydrophobicity and different polarity.[30]

The dissolution and processing of lignocellulosic biomass is a particularly interesting application of ionic liquids (ILs), as they can directly dissolve and fractionate (ligno-)cellulose in an overall less energy intensive process.[31,32] The biomass dissolution capability of ILs is impacted by both their cation and anion, however, current publications suggest that anions have a more significant impact, since they play a role in breaking the many intermolecular hydrogen bonds.[30] Regarding the cation, imidazolium-based ILs were the most successful for the direct dissolution of cellulose, followed by pyridinium- and ammonium-based ones.[33] In addition, increasing the chain length of the cation had a negative influence on the dissolving capabilities of the ILs, as the viscosity increased, and the H-bond acidity decreased. As far as the anion is concerned, dissolving efficiency seems to be determined by the H-acceptor properties of the anion. In general, anions with weak H-bond basicity, for instance, $[BF_4]^-$ and $[PF_6]^-$, could not successfully dissolve cellulose, while ionic liquids based on halide or acetate anions are typically the candidates of choice.[30,34] The growing research on ILs as solvents for lignocellulosic biorefinery also prompted innovations for the extraction of valuable ingredients from plant materials.[35] There are several aspects of ILs that are potentially advantageous for the extraction of high-value compounds: apart from their unique solvent properties and potential environmental benefits, the ability of ILs to dissolve biomass can lead to a better, and higher, yielding access to valuable ingredients embedded in the biopolymers and contribute to a value-added biorefinery.[36,37] However, the recovery of natural products from ionic liquids is often more demanding than the mere extraction: many studies require extensive back-extraction with volatile solvents to actually isolate the valuable ingredients from ILs, thereby rendering the original solvent reduction less significant or even negating it altogether.

The combination of non-volatile polar ILs with volatile non-polar $scCO_2$ has several advantages for extractions, as well as

for catalysis. Since scCO$_2$ is highly soluble in ILs, but ILs cannot dissolve in scCO$_2$, it can easily penetrate the IL-phase. This allows the extraction of compounds from the IL-phase into the scCO$_2$ phase, taken into account that the organic compound of interest is soluble in scCO$_2$. Ultimately they are transported into an extraction vessel in a pure, solvent-free and solid form.[38]

Furthermore, ILs in the presence of CO$_2$ expand their applicability, as their melting point and viscosity decrease, thus, promoting mass transportation.[39] Consequently, the combination of ionic liquids with scCO$_2$ has found application in several catalytic processes, such as hydroformylations, hydrogenations or carboxylations of alkenes in IL-scCO$_2$ biphasic reaction media.[40–43] In the IL-scCO$_2$ reaction systems, the reactants and products are carried by the scCO$_2$ and IL is used as a reaction media.[44,45] Additionally, it is demonstrated that IL-scCO$_2$ biphasic systems avoid cross-contamination of the extracted solute.[38,46]

Until now, IL-based pre-treatment and subsequent SFE (IL-SFE) for natural products has not been described, although ideal conditions arise from the unique properties of both media. Hence, by comparing IL-scCO$_2$ extraction with the utilization of both applications individually or to traditional solvent-extraction, the IL-scCO$_2$ approach is preferable. To begin with, less additional preparation, *e.g.*, filtration of the raw material and consequent evaporation of solvents or separation of IL from the organic solvent is required to obtain a solvent-free and solid extract (Fig. 2). Consequently, there is a lower chance of loss of product or impurities, due to less post processing steps. On the other hand, IL-SFE is performed without additional co-solvents, therefore it reduces further solvent consumption and leads to lower expenses. Ultimately,

if chosen appropriately, the ionic liquid can be recovered and re-used to improve the sustainability of the extraction process.

Recently, an investigation of the extraction of cannabidiol with the aid of ILs has been published; however, isolation of cannabidiol required tedious back-extraction with organic solvents or with an aqueous AgNO$_3$ solution.[47] To the best of our knowledge, no data has been reported thus far regarding a combined extraction process that takes advantage of the complementing properties.

Herein, we present the first application of IL-SFE from industrial hemp of six cannabinoids (Δ^9-THC, THCA, CBD, CBDA, CBG and CBGA). Several parameters during the IL-assisted pre-treatment, such as time, temperature and dilution with H$_2$O, were investigated. In addition, pressure and temperature during SFE were evaluated. Ultimately, the optimized process for 1-ethyl-3-methylimidazolium acetate ([C$_2$mim][OAc]) was additionally performed with choline acetate ([Ch][OAc]) and 1-ethyl-3-methylimidazolium dimethyl phosphate ([C$_2$mim][DMP]) to compare the extraction efficiency of the investigated cannabinoids. In addition, the developed extraction process is complemented by a simple ionic liquid recovering process without the usage of additional organic solvents.

Results and discussion

The focus of this research was the investigation and optimization of various parameters for the extraction of CBD, CBDA, Δ^9-THC, THCA, CBG and CBGA from partially pre-dissolved hemp in various room-temperature ILs with supercritical CO$_2$. The optimization was divided into three successive stages (Scheme 1).
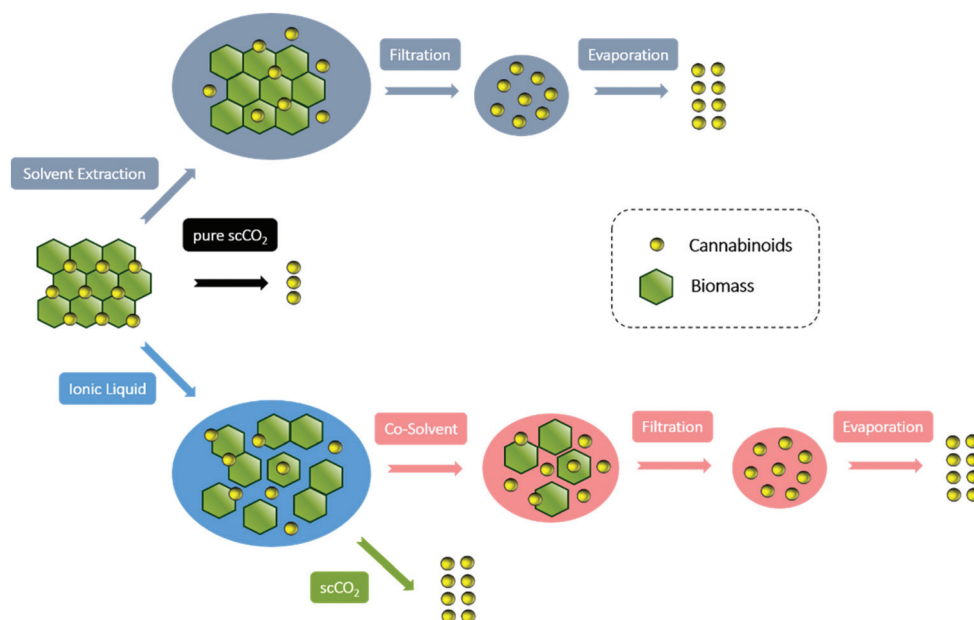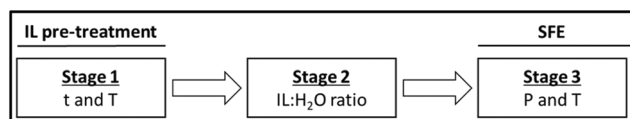

Fig. 2 Conceptualization for the comparison of work up steps and yields of cannabinoids extraction techniques.

IL pre-treatment		SFE
Stage 1 t and T	**Stage 2** IL:H₂O ratio	**Stage 3** P and T

Scheme 1 Successive optimization stages of IL-SFE.

In the first stage, the pre-treatment conditions to digest and partially dissolve hemp using $[C_2mim][OAc]$ before SFE were investigated. The lignocellulosic composition of hemp hurds is reported to contain 43.0% cellulose, 24.4% lignin and 29.0% hemicellulose.[48] ILs are known to dissolve a variety of carbohydrates, *e.g.*, cellulose, by combining strongly basic anions (*e.g.*, Cl^- or OAc^-) with various cations.[49–51] In particular, $[C_2mim][OAc]$ was selected in this study as it was used to pre-treat various lignocellulosic biomasses[52] and it is known to effectively dissolve, hemicellulose[53] and lignin.[54] Furthermore, $[C_2mim][OAc]$ is liquid at room temperature, non-halogenated and miscible with H_2O.

Subsequently, the best extraction conditions of stage 1 were employed in determining the most effective ratio of $[C_2mim][OAc]:H_2O$ during SFE. In the third stage, the previously optimized conditions from the first and second stage were utilized to investigate several combinations of pressure and temperature during SFE. Ultimately, the optimum parameters were employed with two additional ILs, namely $[Ch][OAc]$ and $[C_2mim][DMP]$. Both ILs are liquid at room temperature, non-halogenated and hydrophilic. Moreover, both ILs have been reported for pre-treatment of biomass.[55,56] In addition, the positive rating of choline-based ILs in terms of toxicity and biodegradation renders them ideally suited for natural product extractions.[57,58]

Pre-treatment with ionic liquid (Stage 1)

Herein, the influence of temperature and time for the partial dissolution of *Cannabis sativa* L. in $[C_2mim][OAc]$ before the scCO₂ extraction is evaluated.
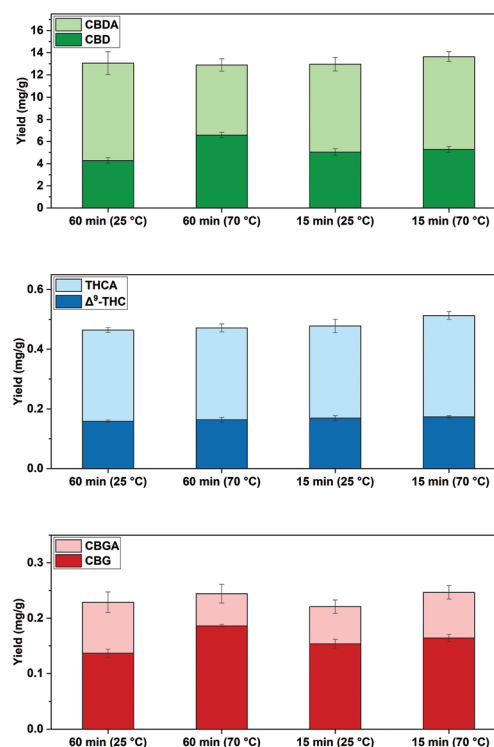
Initially, the conditions to partially dissolve industrial hemp in $[C_2mim][OAc]$ were investigated in experiments 1–4 (Table 1).

Therefore, the pre-treatments were carried out at 25 and 70 °C, each 15 and 60 min, afterwards diluted with H_2O to a ratio of 1 : 2 and subsequently subjected to SFE at 20 MPa and

70 °C. To evaluate the quality of the performed experiments during the development of IL-SFE for hemp the yields of cannabinoids are expressed as the sum of cannabinoid types *e.g.* CBD and CBDA are referred to as $\sum(CBD)$. Analogously $\sum(THC)$ and $\sum(CBG)$ are calculated. All experimental conditions and results for individual cannabinoid yields are shown in the ESI (Tables S1 and S2†).

The cannabinoids CBD and CBDA are predominantly accumulated in industrial hemp compared to THC, THCA, CBG and CBGA, which are considered minor compounds.

The pre-treatment with $[C_2mim][OAc]$ of industrial hemp at 25 °C and 70 °C indicated comparable cannabinoid yields. Increasing the time from 15 to 60 min at 70 °C in exp. 2 led to a small decrease of roughly 5% $\sum(CBD)$ and 8% $\sum(THC)$. However, similar $\sum(CBD)$, but significantly more CBD (6.58 mg g⁻¹) and less CBDA (6.3 mg g⁻¹) at 60 min, was yielded in exp. 2 compared with exp. 4 (15 min), which led to 5.29 mg g⁻¹ CBD and 8.8 mg g⁻¹ CBDA, respectively ($p < 0.05$, Fig. 3, Table S2†). It was reported that an extraction process including $[C_6mim][NTf_2]$ at 60 °C and 50 min leads to high amounts of CBD and that the IL preserves CBD,[47] which correlates with the observations herein. In addition, the decarboxylation of cannabinoids at higher temperatures for longer times has been described before.[8] The IL $[C_6mim][NTf_2]$ was not utilized in this study, as the anion $[NTf_2]^-$ renders it is less suit-

Table 1 Yields of cannabinoids in mg g⁻¹ for the optimization of pre-treatment with $[C_2mim][OAc]$ at different temperatures and time. SFE was performed at 20 MPa and 70 °C with a ratio of $[C_2mim][OAc]:H_2O$ 1 : 2 (Stage 1)

Exp.	t_{Pre}/min	T_{Pre}/°C	$\sum(CBD)$ (mg g⁻¹)	$\sum(THC)$ (mg g⁻¹)	$\sum(CBG)$ (mg g⁻¹)
1	60	25	13.1 ± 0.8[a]	0.464 ± 0.008[b]	0.229 ± 0.011[c]
2	60	70	12.9 ± 0.3[a]	0.471 ± 0.019[b]	0.244 ± 0.014[c]
3	15	25	13.0 ± 0.8[a]	0.48 ± 0.03[b]	0.221 ± 0.019[c]
4	15	70	13.6 ± 0.6[a]	0.513 ± 0.017[b]	0.247 ± 0.017[c]

Mean values with different letters (a, b, c, *etc.*) within the same column are statistically different ($p < 0.05$).

Fig. 3 Comparison of cannabinoid yields (mg g⁻¹) at different pre-treatment temperatures and pre-treatment times with $[C_2mim][OAc]:H_2O$ 1 : 2 and subsequent SFE at 20 MPa and 70 °C, ($n = 3$ ± SD). Experiments refer to Table 1 for Stage 1.

able to dissolve cellulose compared to the basic $[OAc]^-$ or $[DMP]^-$ and similarly, the longer alkyl side chain of the cation would be disadvantageous for this purpose.[59] Ultimately, $[NTf_2]^-$ was not considered for the extraction process, as it is hydrophobic and not mixable with H_2O and thus, not suitable for the IL recovering process shown in here.

A total time of 15 min instead of 60 min seems to be sufficient to release the investigated cannabinoids from the plant tissue with $[C_2mim][OAc]$ and hence, allows a significantly shorter pre-treatment time The highest cannabinoids yields were obtained at 70 °C for 15 min in exp. 4, namely 13.6 mg g^{-1} $\sum$(CBD), 0.513 mg g^{-1} $\sum$(THC) and 0.247 mg g^{-1} $\sum$(CBG) (Table 1).

Ratio of ionic liquid to water (Stage 2)

Optimization of temperature and time during the pre-treatment was performed with a constant ratio of 1 : 2 $[C_2mim][OAc]$: H_2O. Here, the influence of several IL : H_2O ratios was investigated and compared with the sole use of IL as well as pure H_2O in the extraction vessel (Table 2 and Fig. 4).

A decrease of water in the IL : H_2O ratio from 1 : 2 in exp. 4 to 1 : 1 in exp. 5 led to a significant reduction of $\sum$(CBD) as well as $\sum$(THC) yield (Table 2) at 20 MPa and 70 °C. However, the significantly highest yield of CBD (7.45 mg g^{-1}) of all performed IL-SFE was obtained under these conditions in exp. 5 ($p < 0.05$) and additionally, low yields of CBDA (1.09 mg g^{-1}) and no CBGA were extracted (Fig. 4, Table S2†). Therefore, a ratio of 1 : 1 $[C_2mim][OAc]$: H_2O during SFE seems to favour the extraction of neutral CBD and CBG. Recently, it has been discovered that high yields of CBD are extracted by pre-heating hemp and subsequent extraction with supercritical CO_2 combined with EtOH as a modifier.[29] Similar behaviour can be observed under the previously mentioned IL-SFE conditions, without addition of co-solvents.

On the other hand, significantly more $\sum$(CBD) and $\sum$(CBG) ($p < 0.05$) were obtained in exp. 6 by addition of more H_2O to increase the ratio of $[C_2mim][OAc]$: H_2O from 1 : 2 to 1 : 3. The $\sum$(CBD) yield increased by 15% to 15.6 mg g^{-1}, $\sum$(THC) by 6% to 0.542 mg g^{-1} and $\sum$(CBG) by 36% to (0.335 mg g^{-1}) (Table 2). Adding more than 15 wt% H_2O during $[C_2mim][OAc]$ pre-treatment does not allow complete cellulose dissolution,

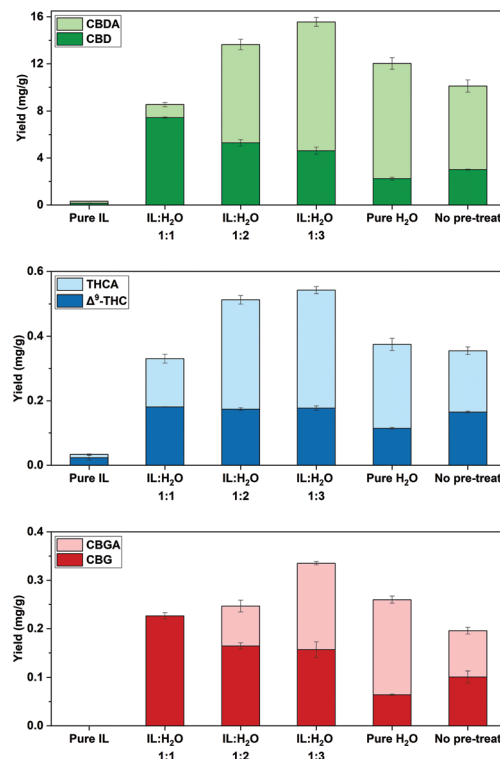**Fig. 4** Cannabinoid yields (mg g^{-1}) for IL-SFE with pure IL and different IL : H_2O ratios, using 15 min of pre-treatment time at 70 °C and for SFE with H_2O (pure H_2O) as well as for scCO$_2$ (no pre-treat). All extractions were performed at 70 °C and 20 MPa; IL = $[C_2mim][OAc]$, ($n = 3 \pm$ SD). Experiments refer to Table 2 for Stage 2.

as reported by Le *et al.* in 2012.[60] Therefore, H_2O was added to the IL after the initial pre-treatment. The addition of H_2O resulted in a reduction of the mixture's viscosity, and thus improved mass transport.[60] It is reported that the viscosity of $[C_2mim][OAc]$ is reduced by 50% when mixed with 10 wt% H_2O and that the IL is less viscous at higher temperatures.[61] Lower viscosity of the IL : H_2O mixture led to higher yields, possibly due to the higher mobility of dissolved cannabinoids and better penetration of scCO$_2$. An increase in carboxylated cannabinoids was observed by adding more water (Fig. 4). Furthermore, water is the only solvent without any negative impacts on the environment. Additionally, it is reported to have low solubility in scCO$_2$[62] and therefore less potential contamination of the extract.

The absence of H_2O during the extraction with scCO$_2$ and $[C_2mim][OAc]$ (pure IL) led to the lowest yields of all SFE in exp. 7 (Table 2 and Fig. 4). Low yields can be a result of the high viscosity of the IL, which leads to less permeability of scCO$_2$ and subsequently lower mass transfer in the extraction. Therefore, dilution with H_2O is essential during the extraction process.

However, the sole extraction with H_2O (pure H_2O) in the absence of $[C_2mim][OAc]$ in exp. 8 compared to exp. 6, leads to a significant reduction of $\sum$(CBD) by 23% to 12.0 mg g^{-1}, $\sum$(THC) by 31% to 0.375 mg g^{-1} and $\sum$(CBG) by 22% to

Table 2 Yields of cannabinoids in mg g^{-1} by investigating the influence of H_2O and $[C_2mim][OAc]$ during SFE with a pre-treatment at 70 °C for 15 min and SFE at 20 MPa and 70 °C (Stage 2)

Exp.	m_{IL}/g	m_{H_2O}/g	$\sum$(CBD) (mg g^{-1})	$\sum$(THC) (mg g^{-1})	$\sum$(CBG) (mg g^{-1})
4	3	6	13.6 ± 0.6^b	0.513 ± 0.017^a	0.247 ± 0.017^b
5	3	3	8.53 ± 0.19^e	0.330 ± 0.014^c	0.226 ± 0.007^{bc}
6	3	9	15.6 ± 0.7^a	0.542 ± 0.016^a	0.335 ± 0.016^a
7	3	—	0.322 ± 0.022^f	0.033 ± 0.006^d	n.d.
8	—	9	12.0 ± 0.6^c	0.375 ± 0.022^b	0.260 ± 0.008^b
9	—	—	10.1 ± 0.5^d	0.355 ± 0.009^{bc}	0.196 ± 0.019^c

Mean values with different letters (a, b, c, *etc.*) within the same column are statistically different ($p < 0.05$).

0.260 mg g^{-1} ($p < 0.05$, Table 2). In particular, the use of H_2O alone tends to yield fewer neutral cannabinoids (Fig. 4), which verifies what has previously been reported; ILs preserve neutral CBD.[47] When comparing exp. 8 with exp. 4, even though the same total quantity of liquid was added in the high-pressure vessel, significantly less yields of $\sum$(CBD) by 12% and $\sum$(THC) by 27% are observed ($p < 0.05$, Table 2, Fig. 4) in the sole water-based SFE extraction. Therefore, a pre-treatment with IL to liberate the cannabinoids from the plant tissue and subsequent dilution with H_2O positively affects the yield.

Ultimately, a reference scCO$_2$ extraction in the absence of both IL and H_2O in exp. 9 (no pre-treatment) yielded 10.1 mg g^{-1} $\sum$(CBD), 0.355 mg g^{-1} $\sum$(THC), 0.196 mg g^{-1} $\sum$(CBG) at 70 °C and 20 MPa (Table 2). Thus, IL-SFE with [C$_2$mim][OAc] : H_2O 1 : 3 in exp. 6 and 1 : 2 in exp. 4, led to significantly higher yields of $\sum$(CBD, THC, CBG) than sole SFE ($p < 0.05$). It has been reported that the cannabinoid yields during SFE can be enhanced by adding EtOH as a modifier.[26,28] In preliminary studies SFE with EtOH as a modifier at different temperatures and vol% EtOH as well as various conventional ethanolic extractions were carried out with another batch of industrial hemp. High yields of the targeted cannabinoids were obtained at 35 °C, 10 MPa and 120 min dynamic extraction with 10 and 20 vol% EtOH. In comparison to the performed conventional extraction, similar $\sum$(THC) yields, but less $\sum$(CBD) and $\sum$(CBG) were yielded ($p < 0.05$, Table S3†). All data is presented in the ESI.†

The addition of EtOH as a co-solvent to IL-SFE would lead to the extraction of both IL and cannabinoids, thus, leading to impurities in the extract. In particular, IL-SFE does not require the use of a co-solvent to obtain cannabinoids in high yields, avoiding further solvent consumption.

Hence, the highest extraction yields were obtained with a IL : H_2O ratio of 1 : 3 in exp. 6, which achieved 15.6 mg g^{-1} $\sum$(CBD), 0.542 mg g^{-1} $\sum$(THC) and 0.335 mg g^{-1} $\sum$(CBG) (Table 2).

SFE extraction parameters – pressure and temperature (Stage 3)

Apart from the optimization of pre-treatment conditions and the ratio of [C$_2$mim][OAc] to H_2O, temperature and pressure during SFE were investigated (Table 3).

Table 3 Yields of cannabinoids (mg g^{-1}) for different temperatures and pressures during SFE. Pre-treatment with [C$_2$mim][OAc] was carried out at 70 °C for 15 min and extracted with a IL : H_2O ratio of 1 : 3 (Stage 3)

Exp.	P_{SFE}/ MPa	T_{SFE}/ °C	$\sum$(CBD) (mg g^{-1})	$\sum$(THC) (mg g^{-1})	$\sum$(CBG) (mg g^{-1})
6	20	70	15.6 ± 0.7[a]	0.542 ± 0.016[a]	0.335 ± 0.016[a]
10	10	70	3.66 ± 0.06[d]	0.0885 ± 0.0026[d]	0.045 ± 0.008[c]
11	15	70	13.00 ± 0.19[c]	0.457 ± 0.005[c]	0.257 ± 0.009[b]
12	30	70	14.7 ± 0.7[ab]	0.500 ± 0.024[ab]	0.36 ± 0.04[a]
13	20	35	14.9 ± 0.7[ab]	0.493 ± 0.014[bc]	0.323 ± 0.009[a]
14	10	35	13.7 ± 0.8[bc]	0.468 ± 0.019[bc]	0.248 ± 0.010[b]

Mean values with different letters (a, b, c, *etc.*) within the same column are statistically different ($p < 0.05$).

Initially, the pressure was reduced from 20 MPa in exp. 6 to 15 MPa in exp. 11 at 70 °C and led to a significant reduction by 17% $\sum$(CBD) to 13.00 mg g^{-1}, 16% $\sum$(THC) to 0.457 mg g^{-1} and 23% $\sum$(CBG) to 0.245 mg g^{-1} ($p < 0.05$, Table 3). After further decreasing the pressure to 10 MPa in exp. 10, a significantly diminished yield of 3.66 mg g^{-1} $\sum$(CBD), 0.0885 mg g^{-1} $\sum$(THC), and 0.045 mg g^{-1} $\sum$(CBG) was observed (Table 3). Even though lower cannabinoid yields were obtained at 10 MPa and 70 °C in exp. 10, the extraction of neutral cannabinoids was favoured (Fig. 5). In literature, sole scCO$_2$ extractions yield neither CBD nor CBDA at 10 MPa at 70 °C for 120 min,[63] but SFE can be improved upon by adding EtOH[26] or by the pre-treatment with IL, as herein reported. In addition, the pressure was increased to 30 MPa at 70 °C in exp. 12, which resulted in comparable yields of $\sum$(CBD, THC, CBG) as IL-SFE at 20 MPa in exp. 6 (Table 3). It can be assumed that 20 MPa at 70 °C are sufficient to extract cannabinoids during IL-SFE.

Furthermore, the temperature was lowered to 35 °C at 20 MPa during SFE in exp. 13. This led to comparable yields of $\sum$(CBD) and $\sum$(CBG), but significantly lower $\sum$(THC) yields (0.493 mg g^{-1}) compared to 70 °C in exp. 6 ($p < 0.05$, Table 3). This corresponds to literature data, where similar yields of $\sum$(CBD) were extracted during SFE at 35 °C and 70 °C at 50 MPa.[63] Lower temperatures are known to reduce the viscosity of H_2O and additionally, have been reported to decrease the viscosity of [C$_2$mim][OAc].[61] Hence, the mixture is less pene-
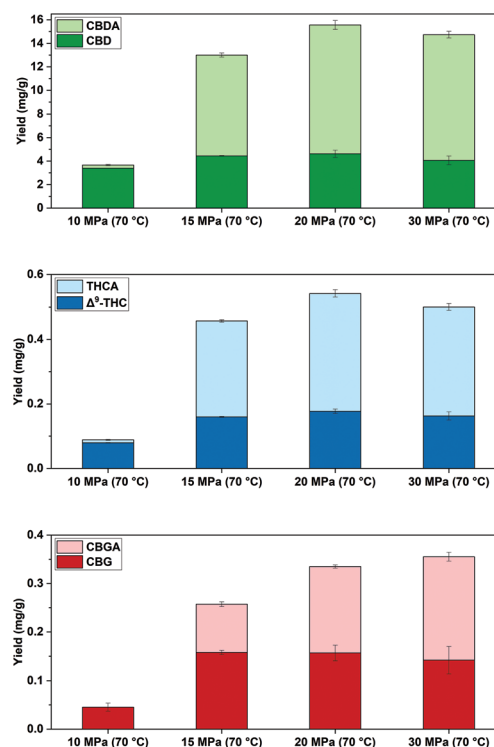
Fig. 5 Cannabinoid yields (mg g^{-1}) at 10, 15, 20 and 30 MPa at 70 °C by scCO$_2$ extraction combined with [C$_2$mim][OAc] : H_2O 1 : 3 and a pre-treatments at 70 °C for 15 min, (n = 3 ± SD). Experiments refer to Table 3 for Stage 3.

trable for $scCO_2$ to extract the target cannabinoids. In comparison of exp. 13 and exp. 6, the yields of decarboxylated cannabinoids decreased significantly (CBD by 28%; Δ^9-THC by 16%; CBG by 33%) and similar yields of THCA and CBDA, but significantly more CBGA by 23% was obtained in exp. 13 ($p < 0.05$, Table S3†).

A further decrease from 20 MPa at 35 °C in exp. 13 to 10 MPa in exp. 14 led to a slight reduction in $\sum$(CBD) by 8% and $\sum$(THC) by 5%, and significant reduction in $\sum$(CBG) by 23% ($p < 0.05$, Table 3). Therefore, a combination of 35 °C and 10 MPa seems to affect the total cannabinoid yield negatively, but increasing the temperature to 70 °C at the same pressure further reduces the yields. Lower CBD and CBDA yields at 10 MPa at 70 °C compared with 35 °C during SFE have been described in literature.[63] Thus, 10 MPa at 70 °C during SFE seem to be unfeasible to extract cannabinoids from industrial hemp. At 20 MPa, the temperature seems to have a minor effect on the total yields of cannabinoids.

Consequently, the optimum cannabinoid yields were obtained at 20 MPa and 70 °C in exp. 6 during supercritical CO_2 extraction.
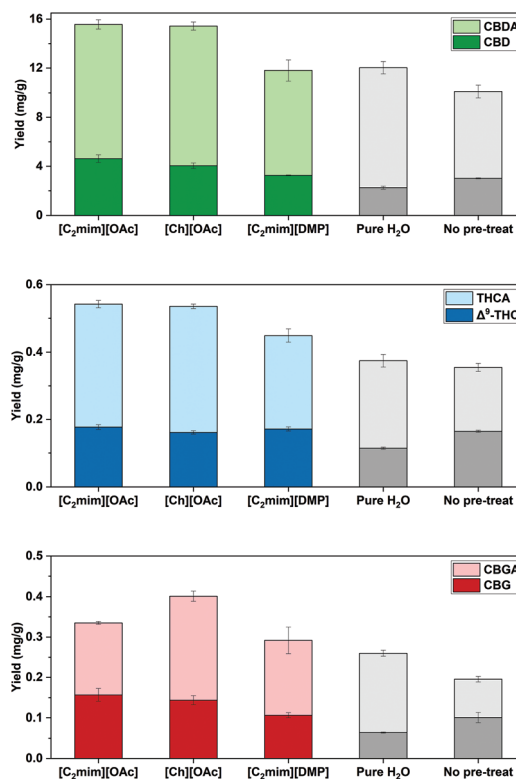
Type of ionic liquid

Two additional ILs, namely, [Ch][OAc] and [C_2mim][DMP], were selected for evaluation alongside [C_2mim][OAc]. The optimized extraction conditions, with a pre-treatment at 70 °C for 15 min and subsequent SFE at 70 °C and 20 MPa with a IL : H_2O ratio of 1 : 3, were additionally applied to these two ILs to observe differences in cannabinoid yields (Table 4 and Fig. 6).

Ionic liquid assisted SFE with [Ch][OAc] in exp. 15 yielded comparable yields of $\sum$(CBD) (15.4 mg g^{-1}) and $\sum$(THC) (0.535 mg g^{-1}), but significantly more $\sum$(CBG) (0.401 mg g^{-1}) than IL-SFE with [C_2mim][OAc] in exp. 6 ($p < 0.05$, Table 4). The change of cation does affect the yields of cannabinoids, however, the role of the cation during the dissolution of lignocellulose structure is not yet fully understood.[64] On the other hand, anions, such as [OAc]$^-$, are described to effectively support the dissolution of cellulose by forming hydrogen bonds.[34]

Fig. 6 Cannabinoids yields (mg g^{-1}) for IL-SFE with [C_2mim][OAc], [Ch][OAc] as well as [C_2mim][DMP] (IL : H_2O 1 : 3), for SFE with H_2O (pure H_2O) and for $scCO_2$ (no pre-treat). All extractions were performed at 70 °C and 20 MPa, ($n = 3 \pm$ SD). Pure H_2O (exp. 8) and $scCO_2$ (no pre-treat) (exp. 9) refer to Table 2. [C_2mim][OAc] (exp. 6), [Ch][OAc] (exp. 15) and [C_2mim][DMP] (exp. 16) refer to Table 4.

To investigate the influence of the anion in IL-SFE of cannabinoids from industrial hemp, the imidazolium-based IL [C_2mim][DMP] was used in exp. 16. This resulted in a significant reduction of $\sum$(CBD) to 11.8 mg g^{-1} and total THC to 0.449 mg g^{-1} compared with the acetate-based ILs in exp. 6 and exp. 15 ($p < 0.05$, Table 4). [C_2mim][DMP] is described as effectively dissolving biomass, but has a high viscosity,[56,65] which could affect the extraction at supercritical conditions, due to the weaker penetration of $scCO_2$. Nonetheless, phosphate based and acetate based IL-SFE yielded higher amounts of $\sum$(CBD, THC, CBG) compared with sole supercritical CO_2 extraction without IL pre-treatment (Fig. 6).

The following mechanism can be proposed for IL-SFE. Firstly, the biomass is partially dissolved by breaking down the lignocellulose structure of the industrial hemp powder. This depends on the anion and cation of the ILs.[34,64] The cannabinoids are released from the plant tissues and the IL possibly stabilizes them.[47] Secondly, the water is added, which reduces the viscosity of the mixture[61] and lowers the solubility of the target cannabinoids. Due to the lower surface tension and higher mobility of cannabinoids, a higher mass transfer between the $scCO_2$ phase and the IL : H_2O phase is generated. As reported the $scCO_2$ dissolves in ILs, however, neither the IL nor the H_2O does dissolve in $scCO_2$.[38,62] Finally, these synergic

Table 4 Yields of cannabinoids (mg g^{-1}) by comparing different ILs and reference extractions in EtOH and H_2O. IL-SFE was performed with a pre-treatment at 70 °C for 15 min, a ratio of 1 : 3 IL : H_2O at 70 °C and 20 MPa during SFE

Exp.	IL or solvent	$\sum$(CBD) (mg g^{-1})	$\sum$(THC) (mg g^{-1})	$\sum$(CBG) (mg g^{-1})
6	[C_2mim][OAc]	15.6 ± 0.7^a	0.542 ± 0.016^a	0.335 ± 0.016^c
15	[Ch][OAc]	15.4 ± 0.5^a	0.535 ± 0.010^{ab}	0.401 ± 0.024^b
16	[C_2mim][DMP]	11.8 ± 0.9^b	0.449 ± 0.025^c	0.292 ± 0.028^c
17^a	EtOH for 2 h	15.4 ± 0.4^a	0.498 ± 0.018^b	0.452 ± 0.019^a
18^a	EtOH for 24 h	14.84 ± 0.15^a	0.447 ± 0.005^c	0.440 ± 0.004^{ab}
19^a	H_2O for 2 h	1.6 ± 0.3^c	0.057 ± 0.012^d	0.031 ± 0.007^d

Mean values with different letters (a, b, c, *etc.*) within the same column are statistically different ($p < 0.05$). ^{a} At 70 °C.

effects allow the scCO$_2$ to extract the targeted cannabinoids, due to better solubility in the supercritical phase without contaminating it with IL or H$_2$O. Thus, no further organic solvents are necessary to purify the compounds from the IL phase and consequently, no additional work up is needed to obtain a solid and solvent free product (Fig. 2).

Ultimately, IL-SFE was compared with reference solvent extraction (exp. 17–19). Ethanol is one of the most commonly used solvents to extract cannabinoids.[66] Herein, a conventional extraction for 2 h, at 70 °C, with EtOH in exp. 17, sufficiently extracted the investigated cannabinoids; however, employing H$_2$O in exp. 19 alone under the same conditions, low yields of cannabinoids were obtained (Table 4). A control extraction in EtOH for 24 h was carried out in exp. 18 to investigate the influence of longer extraction times. Longer times at high temperatures seem to degrade carboxylated cannabinoids significantly, reducing CBDA by 52%, THCA by 65% and CBGA by 53% ($p < 0.05$, Table S2†). The decarboxylation of cannabinoic acids at high temperatures for longer times is described in literature.[8] However, it can be reported that the degradation over time does not affect the overall cannabinoid yields.

By comparing the two-hour ethanolic extraction (exp. 17) with acetate based ionic liquid-SFE, several differences can be observed. Firstly, the yields of ∑(CBD) by SFE with [C$_2$mim][OAc] in exp. 6 and [Ch][OAc] in exp. 15 are slightly higher but comparable to the 2 h ethanolic extraction (Table 4). Secondly, significantly more ∑(THC) with [C$_2$mim][OAc] compared with the two-hour reference extraction with EtOH is obtained. Ultimately, the reference extraction yielded more ∑(CBG) than IL-SFE using [Ch][OAc] or [C$_2$mim][OAc] ($p < 0.05$, Table 4). Hence, the results underline the importance of appropriately selecting the IL cation and anion, as well as the optimal extraction parameters for IL-SFE to extract cannabinoids from industrial hemp. Ultimately, [Ch][OAc] based SFE yields high amounts of the investigated cannabinoids and also provides environmental and economic benefits. Not only is [Ch][OAc] biodegradable, but it is also considered relatively cheap (88 € for 25 g), easy to synthesize, as well as less toxic compared to other ionic liquids.[57,58,67,68] Furthermore, no co-solvents are applied during IL-SFE, which avoids additional solvent consumption and consequently leads to a purer, solid extract (Fig. S2†). Ultimately, all three ILs were purified without any additional use of organic solvents. Neither water nor significant impurities were detected by NMR spectroscopic analysis for the purified ILs (Table S4 and Fig. S5–S7†) and thus, can be re-used for IL-SFE.

Conclusions

Herein, we report a novel IL-based dynamic supercritical CO$_2$ extraction process for the isolation of cannabinoids from *Cannabis sativa* L. The investigation showed that 15 min at 70 °C pre-treatment of hemp with [C$_2$mim][OAc] and [Ch][OAc], dilution of IL with H$_2$O (1 : 3) and ultimately, scCO$_2$ extraction at 20 MPa and 70 °C for 2 h, led to high yields of the investigated cannabinoids. Acetate-based ILs resulted in higher yields of cannabinoids compared to phosphate-based ILs. In addition, IL-SFE with [C$_2$mim][OAc] yielded significantly more ∑(THC) than conventional extraction with EtOH. Hence, the type of IL is of great importance and affects the cannabinoid yield significantly. However, not only the type of IL needs to be selected carefully, also the SFE parameters. In dependence of various parameters, e.g. IL pre-treatment temperature or the ratio of IL : H$_2$O during SFE, it is possible to adjust the proportion of carboxylated and decarboxylated cannabinoids in the extracts. In addition, IL-SFE allows extracting cannabinoids in highest yields and, therefore, it can be reported as a novel competitive alternative to traditional extraction techniques or supercritical fluid extraction with co-solvents. Ultimately, the ILs can be recycled without additional usage of further organic solvents to reduce costs and improve the sustainability of the process. IL-SFE offers the opportunity to extract secondary metabolites from different natural sources without volatile organic solvents and the presented process has great potential for future industrial applications.

Experimental

Plant material

The type III chemovar Futura 75 was cultivated in Austria, in the fields of Biobloom (Apetlon, Austria, 7°41′23.4″N 16°56′26.7″E), in September 2020. After the harvest, the plants (flowers, leaves and stems) were stored under mild conditions at 40 °C for 14 h. The samples were milled with a Fritsch Universal Pulverisette 19 mill through a 2 mm sieve (Fritsch, Oberstein, Germany). The dry matter was 94.73 ± 0.05 wt% (n = 3). A second batch of the same industrial hemp harvested in 2019 was used for the preliminary experiments, mentioned in section Results and discussion. The dry matter was 93.68 ± 0.03 wt% (n = 3). The hemp raw material was stored in the dark, at −20 °C, between experiments.

Ionic liquid-supercritical fluid extraction

For pre-treatment, a high-pressure vessel of approximately 50 mL (EV-3), produced by Jasco (Jasco Corporation, Tokyo, Japan), containing one input and one output connections on the lid, was used. The batch reactor was charged with 0.20 g milled hemp and 3 g of IL. [C$_2$mim][OAc] (≥90%) was purchased from BASF (Ludwigshafen am Rhein, Germany), [Ch][OAc] (98%) from IoLiTec (Heilbronn, Germany) and [C$_2$mim][DMP] (98%) from ABCR (Karlsruhe, Germany). Pre-treatment optimization was performed for 15 min and 60 min, at 25 °C and 70 °C, respectively, with [C$_2$mim][OAc]. Furthermore, [C$_2$mim][OAc] was diluted with different amounts of H$_2$O (filtered through a Milli-Q ion exchange system) after the pre-treatment to evaluate the effect on extraction efficiency. Therefore, 3 g of IL were mixed with 3 g, 6 g and 9 g of H$_2$O and stirred for 10 min before SFE. In addition, extraction purely with [C$_2$mim][OAc], without the addition of water, was tested.
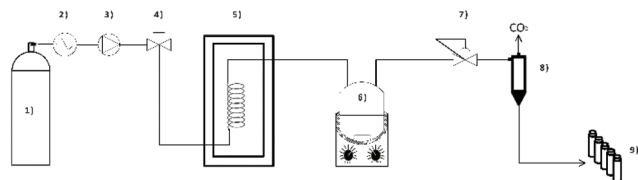
Fig. 7 General setup for the dynamic extraction of cannabinoids using IL-SFE. (1) Liquid CO_2 supply, (2) chiller/cooling system, (3) CO_2 pump, (4) manually operated valve, (5) thermostated oven with preheating coil, (6) high pressure vessel placed on a thermostated stirrer, (7) back pressure regulator (BPR), (8) gas–liquid separator, and (9) fraction collector.

The SFE setup is presented in Fig. 7. All extractions were performed with a $scCO_2$ device manufactured by Jasco (Jasco Corporation, Tokyo, Japan). Liquid CO_2 (>99.995% purity; with ascension pipe; Messer GmbH, Vienna, Austria) was pressurized by two CO_2-pumps (PU-2086, Jasco Corporation, Tokyo, Japan) with cooled heads (CF40, JULABO GmbH, Seelbach, Germany). An oven (CO-2060, Jasco Corporation, Tokyo, Japan) with a heating coil was used and was thermostated to the desired temperature. The vessel containing the IL pre-treated hemp was placed on a heating mantle set to a certain temperature and a stirring rate of 500 rpm and, subsequently, connected to the supercritical carbon dioxide ($scCO_2$) device. A back-pressure regulator (BP-2080, Jasco Corporation, Tokyo, Japan), a gas/liquid separator (HC-2086-01, Jasco Corporation, Tokyo, Japan), and a product collector (SCF-Vch-Bp, Jasco Corporation, Tokyo, Japan) were used to obtain the extracts.

The conditions employed for the SFE of cannabinoids were based on literature data[63,69] and adapted for our purposes. The CO_2 flow rate, the static extraction and the dynamic extraction were set to 5.0 mL min^{-1}, 30 min and 120 min, respectively. Different variables were evaluated during SFE, e.g., oven temperature, heating mantle temperature (35 °C and 70 °C, respectively) and pressure (10 MPa, 15 MPa, 20 MPa and 30 MPa), using [C_2mim][OAc]. Ultimately, the optimized conditions were applied to [Ch][OAc] and [C_2mim][DMP]. After each extraction, the extracts were collected and diluted to a defined volume with ethanol and prepared for analysis by HPLC. EtOH was purchased from Chem-Lab (Zedelgem, Belgium, abs.).

Solvent-based extraction

For comparison, conventional solvent extractions were performed in 30 mL Teflon screw cap vials. The hemp quantity used in each extraction was 0.2 g. Two extractions were performed in triplicate using 2 mL solvent, more precisely, H_2O and EtOH, for 2 h at 70 °C and a third one, also in triplicate, using 10 mL EtOH for 24 h and 70 °C.[70]

Ionic liquid recovering

After extraction, the $scCO_2$ device was depressurized, the metallic extraction reactor was disconnected and brought to room temperature. The IL–water–hemp mixture (Fig. S3†) was filtered to remove hemp particles, the water was evaporated in vacuo and the remaining ionic liquid was dried under vacuum (0.65 mbar) for 24 h. Afterwards 20 mg of purified IL (Fig. S4†) were dissolved in chloroform-d_3 (Sigma Aldrich, St Louis, USA) and a ^{1}H-NMR was recorded with a 400 MHz Bruker Advanced Ultra Shield 400 spectrometer (Bruker, Billerica, USA). Spectroscopic data and NMR spectra are given in the ESI (Table S4 and Fig. S5–7†)

Cannabinoid quantification

The determination of CBDA, CBD, CBGA, CBG, THCA, Δ^9-THC, was carried out on a High-Performance Liquid Chromatography (HPLC) in a Dionex UltiMate© RSLC System, with DAD-3000RS Photodiode Array Detector (Thermo Scientific, Germering, Germany), on a Dionex Acclaim™ RSLC 120 C18 (2.2 µm, 120 Å, 2.1 × 150 mm, Bonded Silica Products: no. 01425071, Thermo Scientific, Germering, Germany). A mobile phase flow rate of 0.2 mL min^{-1} was employed and the oven temperature was set to 25 °C. As a mobile phase, H_2O with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) were used. The following gradient was carried out: 2 min of pre-equilibration at 70% B, 6 min hold at 70% B, 6 min from 70% B to 77% B, 18 min hold at 77% B, 0.5 min from 77% B to 95% B, 1.5 min at 95% B, 0.5 min from 95% B to 70% B, and 5 min at 70% B.[71] Acetonitrile was purchased from VWR Chemicals (Radnor, PA, USA) and formic acid from Merck (Darmstadt, Germany). All solvents for HPLC were of analytical grade.

The cannabinoid standards CBD, CBDA, THCA, Δ^9-THC, CBG and CBGA were provided by Medical Cannabinoids Research and Analysis GmbH (Brunn am Gebirge, Austria) in the course of previous joint research. A mixed cannabinoid stock solution (1 mg mL^{-1}) in MeOH of the investigated cannabinoids diluted for calibration.

Statistical analysis

Statistical data analysis was performed with Origin 2021. One-way ANOVA for multiple groups, followed by Tukey honestly significant difference (HSD) post hoc test at the 0.05 significance level, was carried out.

Addendum

The authors would like to point out that the focus of this study was the extraction of cannabinoids as a class, not THC specifically. Any THC extraction is purely incidental, and bound to be negligible, given that industrial hemp was used, which in the EU must have a THC content not in excess of 0.2%.

The relevant EU law can be perused under: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32013R1307&from=de.

In particular, we refer to Article 32, paragraph 6.

Additionally, the authors do hold a licence to for the purposes of research, in accordance with Austrian law, available under:

https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10011053.

Author contributions

C.K. & A.S.M.: conceived the research, designed and performed the experiments, analysed the data, wrote the original draft, edited and reviewed the manuscript. M.S.: supervised the research, edited and reviewed the manuscript. K.S. & H.H.: conceived and supervised the research, designed the experiments, edited and reviewed the manuscript.

Conflicts of 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 article.

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