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ORIGINAL ARTICLE

Development of a solvent screening methodology for cannabinoid recovery from a wax by-product via recrystallization

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Abstract
Wax by-products from commercial *Cannabis* processing, containing between 41 and 46% (w/w) cannabinoids, are currently underutilized due to lack of research done, and significant value can be added through recovery of the cannabinoids. The cannabinoids are aimed to be recovered via a novel solvent-assisted recrystallization technology, and this study provides a robust solvent screening methodology for recovery of the cannabinoids for this technology. Solvents were screened based on their relative polarity, boiling point temperature, and safety of use. Further criteria such as reactivity toward wax compounds were implemented through evaluation of the Kamlet-Taft parameters of potential solvents. Solubility predictions of the wax in different solvents were done using the Hansen solubility parameters (HSP). The methodology was tested on a set of 73 commonly used solvents identifying five suitable solvents, which were 1,2-dimethoxyethane, 3-pentanone, ethyl acetate, methyl acetate, and methyl tert-butyl ether. The identified solvents were tested for use in recrystallization and were found to be suitable, validating the use of the developed methodology.

Keywords *Cannabis* · Solvent screening · Hansen solubility parameters · Plant wax

1 Introduction

Commercial cannabinoid extraction generally follows the route of primary solid–liquid extraction from plant material with ethanol, followed by a winterization step to precipitate co-extracted plant waxes [1]. The precipitated waxes are then removed from the primary stream through filtration as the presence of lipophilic compounds in the extracts negatively affects the performance of the various downstream distillation stages [2]. The utilization of the *Cannabis* wax holds significant potential to unlock additional value from by-products as 5%–10% (w/w) of total raw material input can be recovered as by-product generated during cannabidiol (CBD) isolation [3].

Currently, the wax by-product is underutilized due to a lack of viable cannabinoid recovery methods, and literature regarding characterization of the wax is sparse. An additional process step is proposed to be added for the recovery of the cannabinoids from the wax, as shown by Fig. 1, from where the recovered cannabinoids are set to be reintroduced to the overhead stream before the primary distillation stage. Primary extraction generally implements the use of ethanol as the working solvent, and after winterization and solvent evaporation, the resulting concentrate can have a cannabinoid content of up to 70% (w/w) [4]. The fractionation of plant waxes is often achieved through the use of a solvent, typically liquid substances in which other substances (solutes) can dissolve and be recovered unchanged through the removal of the solvent [4, 5]. Selective dissolution of solutes in the solvent results in the separation of compounds based on solubility [6]. Dissolution of a compound in a solvent occurs when the attraction forces between the solvent and solute exceeds the interaction forces of solvent–solvent and solute–solute [7].

As these forces are dependent on the physical properties of the solvent, the selection of a suitable solvent is critical

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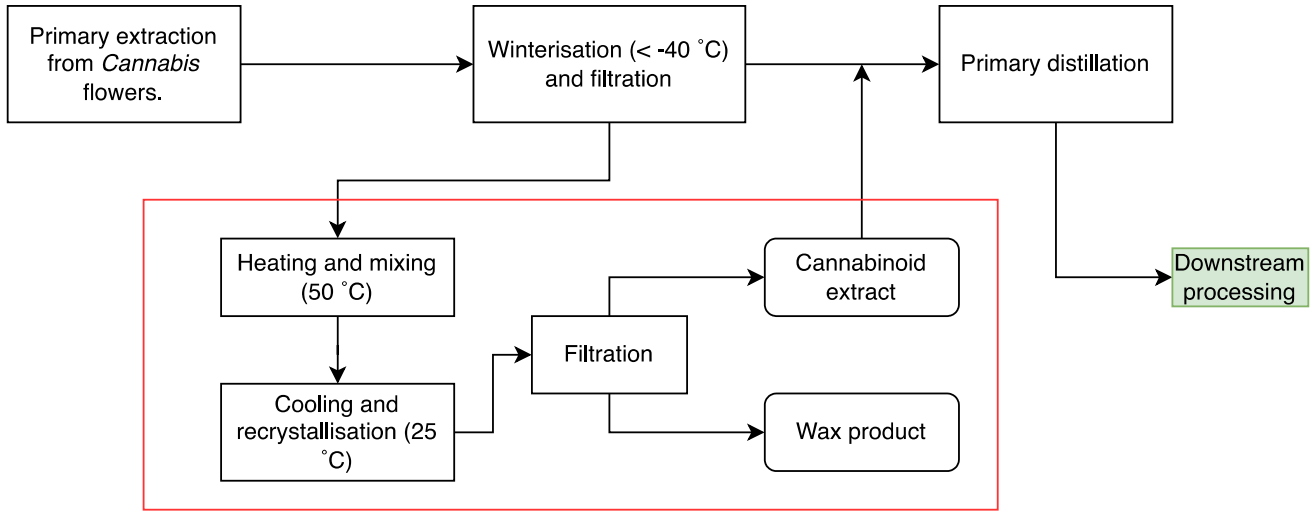

Fig. 1 Process flow diagram of *Cannabis* processing with an additional cannabinoid recovery stage included and emphasized in red

for effective recovery of cannabinoids from the wax. Specific solvent–solute interactions are often defined according to the so-called solvent polarity. Practically, solvent polarity refers to the overall solvation capability of a solvent for a solute and is dependent on all possible intermolecular forces between the solvent and solute [7]. One set of parameters often used to predict solvent–solute interactions is the Kamlet-Taft parameters [6, 7]. The set of solvatochromic parameters for α, β, and π^* were developed by Kamlet and Taft, where α is the hydrogen bond donating ability, β is the hydrogen bond accepting ability, and π^* is the polarizability of a substance [8]. These parameters can be used as a prediction tool for solvent–solute interactions such as reactivity and solubilization. Another set of parameters that can be used to predict solubility of a solute in a solvent is the Hansen solubility parameters (HSP). This set of parameters recognizes that the total cohesion energy consists of several individual contributions arising from the dispersion, dipole–dipole, and hydrogen bonding forces. The total cohesion energy is equal to the sum of these three major interaction energies, with a solubility parameter allocated to each contribution [9].

These parameters represent a sphere in a 3D space, referred to as the Hansen sphere. The dimensions of a given sphere are the Hansen solubility parameters (HSP), and specific solvent/solute interactions and solubility can be predicted through calculation of the distance between the spheres of a solvent and solute.

This study is aimed at developing a thorough solvent screening methodology for the purpose of non-reactive fractionation of the *Cannabis* wax by-product via a novel recrystallization technology, which has demonstrated cannabinoid recoveries of above 75% (w/w) using pure ethyl acetate as the solvent. During fractionation, it is aimed to recover the valuable oil phase entrapped in the wax-matrix, which contains a high concentration of cannabinoids. The methodology developed in this study will contribute to fill the gap in literature regarding valorization of the wax by-product.

2 Materials and methods

The entrapped cannabinoids were aimed to be recovered through a newly developed recrystallization method using a solvent to assist in the separation of the target compounds (i.e., cannabinoids) from a wax by-product removed during the initial stages of *Cannabis* processing. This involved a stage where the wax is heated to 50 °C and mixed with the solvent of choice to allow for dissolution of the wax, followed by cooling of the mixture to 25 °C where the wax components would recrystallize out of solution. The precipitated waxes are then removed via filtration, and the cannabinoid containing extract is recovered for further processing.

Quantitative analysis was done on the wax by gas chromatography (GC) coupled with mass spectrometry (MS), and the major fractions identified were cannabinoids, n-alkanes, and free fatty acids. Other abundant fractions identified in the wax were fatty alcohols and sterols. The cannabinoid fraction was entrapped in the crystalline wax structure along with residual ethanol from the overhead process. A secondary aim of cannabinoid recovery from the wax was to produce a de-oiled wax (i.e., cannabinoid free) suitable for further refining into a valuable product. The recrystallization method required both dissolution and melting of the lipophilic compounds in order to free the entrapped cannabinoids. From these aims and requirements, the following set of guidelines was proposed to guide the solvent selection methodology:

1. The solvent-by-product system should be non-reactive, to prevent conversion of wax components into lower-value products, or into contaminants that may be carried over into the cannabinoid-rich fraction
2. Carry-over of lipophilic compounds into the solvent phase needed to be minimal to limit the re-introduction of lipophilic compounds to the overhead CBD isolation pathway and to maximize the fraction of de-oiled wax
3. The boiling point temperature of a solvent needed to be higher than the melting point of the wax (46 °C)
4. Selective solubility of the lipophilic compounds needed to be high at 50 °C to ensure dissolution, but low at temperatures of 25 °C and lower to ensure recrystallization
5. The chosen solvent should facilitate cost-effective downstream recovery of the cannabinoids once waxes have been recrystallized from the liquid phase
6. Feasible solvents should not be hazardous to operators or potential users of the cannabinoid isolates

This study investigated several common solvent parameters and their effects on the recovery of cannabinoids to determine suitable criteria for solvent selection. As a first screening, the solubility of the wax was determined theoretically with HSP followed by the evaluation of the relative polarity (RP) of several solvents. RP is a measure of the degree of interaction of the solvent with various polar test solutes and is an experimentally determined value based on a solvent's solvation ability, relative to that of pure water. Subsequent parameters used as screening tools in order of evaluation were boiling point temperature (T_b), safety of use, and Kamlet-Taft parameters for acidity, basicity, and polarity (α, β, and π^*) [4, 5, 7, 9, 10].

2.1 Solubility predictions

The use of HSP was implemented to predict the solubility of the wax in different solvents. Literature data on the HSP of 25 commonly used solvents were collected [11]. The Hansen sphere is represented by Eq. 1. To determine the HSP of the wax, solubility parameters for the most abundant lipophilic compounds as determined through GC were collected [12]. The relative abundance of each compound was used to determine a weighted average for each of the Hansen parameters to represent the wax.

$$\delta_{HSP}^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (1)$$

In Eq. 1, δ_{HSP} is the total solubility parameter and is equal to the sum of the squares of the Hansen components for dispersion-, polar-, and hydrogen bonding forces, denoted as D, P, and H, respectively. Determination of the radius of the solubility sphere (R_0) for the wax is a complex undertaking and fell beyond the scope of this study. The aim of this work was to demonstrate the use of HSP to predict solubility to be a valid, preliminary screening tool when it comes to solvent selection. Therefore, the R_0 value for *Cannabis* wax, based on literate values of other plant waxes (R_0 of 3.8 – 4.5) [12], was chosen to be 4.2 and assumed to be a fair approximation to enable initial screening of different solvents. The HSP parameters for the *Cannabis* wax was based on the compositional results of wax as determined by GC–MS analysis. The HSP of the most abundant lipophilic compounds was collected and used to calculate a mass-weighted average HSP for the *Cannabis* wax. The distance (Ra) between each solvent and the wax was calculated with Eq. 2, which is an empirical model developed from plots of experimental data, where the constant 4 was found to correctly represent the solubility data as a sphere encompassing solvents that are able to dissolve the solute at the specified temperature [9]. Equation 2 was used to determine the relative energy difference (RED) value between each solvent and the wax, which is a ratio of cohesion energies and is determined by dividing Ra by R_0 [9]. RED values > 1 were indicative of solvents falling outside the solubility sphere of the wax and no dissolution would occur. RED values < 1 meant that solvents fell within the solubility sphere and would dissolve the wax. In Eq. 2, subscripts D, P, and H indicate the contributions of dispersion, dipole–dipole, and hydrogen-bonding forces, respectively.

$$(Ra)^2 = 4\left(\delta_{D,\text{solv}} - \delta_{D,\text{wax}}\right)^2 + \left(\delta_{P,\text{solv}} - \delta_{P,\text{wax}}\right)^2 + \left(\delta_{H,\text{solv}} - \delta_{H,\text{wax}}\right)^2 \quad (2)$$

2.2 Experimental validation

To justify the limitations implemented on the various parameters, potential solvents were tested for use in the recovery of cannabinoids through recrystallization. This involved using a specific test solvent to both dilute the wax during heating and also as a washing solvent during filtering. 5 g of wax by-product was mixed with the test solvent at a ratio of 3:1 (w solvent/w wax) and heated to 50 °C. After near complete dissolution of the wax, the mixture was allowed to cool under ambient conditions to 25 °C, during which the wax components crystallized out of solution. The cooled mixture was then filtered using a vacuum filter, and additional solvent was used for washing, at a ratio of 2:1 (w solvent/w wax). The suitability of a solvent was evaluated by visual inspection of wax dissolution at 50 °C, reactivity, recrystallization below 25 °C, and wax carry-over during filtration.

3 Results and discussions

In the aim to recover entrained cannabinoids from the wax by-product, generated during the initial extraction stage of cannabinoids from *Cannabis*, the use of a solvent

was implemented to assist in the fractionation of the wax. The wax was fractionated through temperature controlled recrystallization of the lipophilic compounds that was then separated from the entrapped solvent phase through filtering. A critical aspect regarding the development of the recrystallization methodology was to further develop a solvent screening process to be used for the identification of solvents suitable for use in this method.

The recrystallization method was dependent on two physical changes that needed to occur as to free the cannabinoids from the wax matrix. First, to ensure that the cannabinoids were able to come into contact with and dissolve into the added solvent, the waxes needed to be heated to above their melting point (approximately 46 °C). Near complete dissolution of the waxes at the elevated temperature ensured that the entrapped cannabinoid fraction was freed and allowed to partition into the solvent phase. The other physical constraint was that at the recrystallization temperature (< 25 °C), the dissolution of wax in the solvent should be limited, while retaining the dissolved cannabinoids in the solvent. Therefore, the waxes would recrystallize out of the cannabinoid-rich solvent phase which would allow for the solidified waxes to be recovered through physical means, such as filtration.

These two physical constraints placed limitations on the solvation ability and other physical properties such as boiling point temperature of proposed solvents. The limitations on solvation ability of solvents were investigated by predictive solubility parameters and the evaluation of a solvent's polarity on dissolution of the wax. Constraints on boiling point temperatures were fixed by the physical requirements of the recrystallization method and cost of downstream refinement of the recovered cannabinoids. Finally, reactivity and the safety of use of proposed solvents were used as final selection criteria to remove unsuitable solvents from the list of possibilities. A list consisting of commonly used solvents for industrial processes was compiled [5, 9, 13], containing 73 solvents as listed in Table 1. The methodology developed in this study was applied to the list, to identify potentially suitable solvents.

3.1 Solubility predictions

The effect of polarity on the solubility of the wax was demonstrated through the use of HSP. The HSP of several solvents commonly used in industry for plant wax fractionation was collected [12]. Evaluated solvents were chosen based on functional groups and available solubility data, where the variation in compound classes was done

Table 1 Solvents commonly used in industry, as evaluated for the purpose of treating the *Cannabis* wax by-product

1-Butanol	Benzyl alcohol	Glycerin
1-Heptanol	Carbon disulfide	Heptane
1-Hexanol	Carbon tetrachloride	Hexafluoroisopropanol
1-Octanol	Chlorobenzene	Hexane
1-Pentanol	Chloroform	HMPT
1-Propanol	Cyclohexane	i-Butanol
1,1-Dichloroethane	Cyclohexanol	Isopropanol
1,2-Dichloroethane	Cyclohexanone	Methanol
1,4-Dioxane	Di-n-butylphthalate	Methyl acetate
2-Aminoethanol	Dichloromethane	Methyl *t*-butyl ether (MTBE)
2-Butanol	Diethylamine	Methylene chloride
2-Butanone	Diethylene glycol	Methyl ethyl ketone
2-Pentanol	Diglyme	N,N-dimethylaniline
2-Pentanone	Dimethoxyethane (glyme)	ODCB (orthodichlorobenzene)
2-Propanol	Dimethylformamide (DMF)	p-Xylene
3-Pentanol	Dimethyl phthalate	Pentane
3-Pentanone	Dimethyl sulfoxide (DMSO)	Pyridine
Acetic acid	Dioxane	Sulfolane
Acetone	DMPU	t-Butyl alcohol
Acetonitrile	Ethanol	Tetrahydrofuran (THF)
Acetyl acetone	Ether	Toluene
Aniline	Ethyl acetate	Trifluoroethanol
Anisole	Ethyl acetoacetate	Water
Benzene	Ethyl benzoate	
Benzonitrile	Ethylene glycol	

as this resulted in variation of the magnitude of the three contributions (dispersion, dipole–dipole, and hydrogen bonding). These parameters were used to predict the solubility of the wax in each solvent at 25 °C. The solvents with their respective HSP are given in Table 2. The wax was characterized with regard to its major components by GC–MS analysis. To determine the HSP of *Cannabis* wax, HSP data for individual components in plant waxes were collected and individual contributions were assigned according to the component's relative abundance in the *Cannabis* wax. The mass weighted averages were calculated as 16.8, 1.4, and 2.8 for δ_P, δ_D, and δ_H, respectively. The total HSP (δ_{HSP}) for *Cannabis* wax was subsequently calculated as 16.8 $(1/MPa)^{1/2}$.

The values of δ_P, δ_D, and δ_H were used as coordinates for points on a three-dimensional space, graphically displayed in Fig. 2. For the point representing the HSP of the wax, a sphere of solubility with radius R_o was used to predict which solvents would dissolve the wax at 25 °C. All solvents with points falling within the sphere of solubility would theoretically dissolve the wax.

In principle, an increase in temperature would result in changes in the three contributions of the HSP (δ_P, δ_D, and δ_H), and therefore, change the coordinates of the solvent and the solute spheres. This change in coordinates for the different spheres can result in solvent spheres moving into the solubility region (RED < 1) which initially fell outside (RED > 1), i.e., at higher temperatures, these solvents are able to dissolve the wax. To be able to predict the effect of temperature on solubility using HSP, accurate values of the thermal expansion coefficients of different compounds are needed, which was not readily available for the lipophilic compounds. Further work is proposed to be done on solubility prediction of the wax at higher temperatures, using HSP. The predictions as done in this project were used to identify solvents which would dissolve the lipophilic compounds at 25 °C and would therefore not be suitable for use in cannabinoid recovery through recrystallization.

Two solvents fell within the solubility sphere of the wax by-product, which were n-hexane and cyclohexane. Therefore, at 25 °C, only these two solvents would dissolve the wax by-product. This prediction was validated through testing with hexane as discussed in the following section.

Dissolution of the waxes at 25 °C is unfavorable as it would prevent the lipophilic compounds from recrystallizing out of solution and therefore separation of the cannabinoids from the wax would not be possible. Both these solvents were non-polar, implying increased solubility of the wax in solvents with decreasing polarity. To ensure recrystallization of the lipophilic compounds, a lower limit on the polarity of a potential solvent is therefore required as well as further investigation into the effects of polarity on the suitability of a solvent for cannabinoid recovery.

Table 2 HSP of commonly used solvents for fractionation of plant waxes at 25 °C [11, 12]

Solvent	1/MPa 1/2			
	δ_{HSP}	δ_D	δ_P	δ_H
Alkanes				
n-Butane	14.1	14.1	0	0
n-Pentane	14.5	14.5	0	0
n-Hexane	14.9	14.9	0	0
Cyclohexane	16.8	16.8	0	0.2
Halohydrocarbons				
Chloromethane	17	15.3	6.1	3.9
Chloroform	19	17.8	3.1	5.7
1,1 Dichloroethane	18.5	16.6	8.2	0.4
o-Dichlorobenzene	20.5	19.2	6.3	3.3
Ethers				
Tetrahydrofuran	19.4	16.8	5.7	8
1,4 Dioxane	20.5	19	1.8	7.4
Diethyl ether	15.8	14.5	2.9	5.1
Ketones				
Acetone	20	15.5	10.4	7
Methyl ethyl ketone	19	16	9	5.1
Cyclohexanone	19.6	17.8	6.3	5.1
Esters				
Methyl acetate	18.7	15.5	7.2	7.6
Ethyl acetate	18.1	15.8	5.3	7.2
Nitrogen compounds				
Nitrobenzene	22.2	20	8.6	4.1
Pyridine	21.8	19	8.8	5.9
Aniline	22.6	19.4	5.1	10
Alcohols				
Methanol	29.6	15.1	12.3	22.3
Ethanol	26.5	15.8	8.8	19.4
Acids				
Formic acid	24.9	14.3	11.9	16.6
Acetic acid	21.4	14.5	8	13.5
Oleic acid	15.6	14.3	3.1	14.3
Other				
Water	47.8	15.6	16	42.3

The sensitivity of the results to the chosen R_0 value was investigated by varying the value between 3.8 and 4.5, based of literature values of other plant waxes. At a R_0 of 3.8, only cyclohexane fell within the solubility sphere and therefore was able to dissolve the wax at 25 °C. At a R_0 of 4.5, n-hexane, cyclohexane, and diethyl ether fell within the solubility sphere. This showed that the solubility analysis was sensitive toward the R_0 value, as is expected, but all solvents that fell within the solubility sphere in this R_0 range (3.8 – 4.5) had very low relative polarities. This further validated investigation into the effects of polarity on the suitability of a potential solvent, and that there will need to be limitations imposed on the polarity of a solvent to prevent dissolution of the waxes at 25 °C.

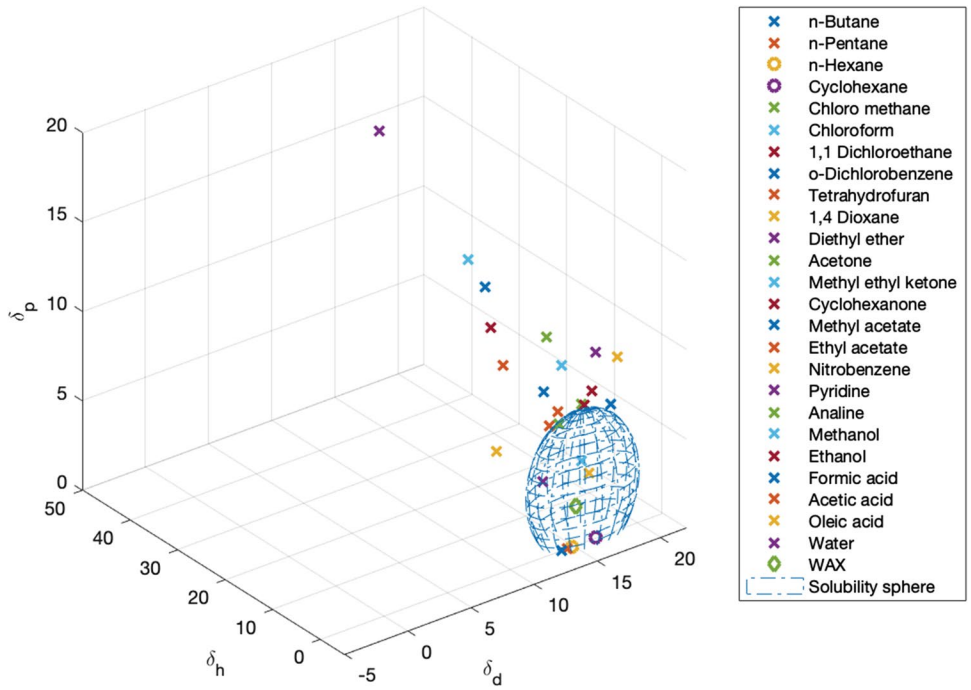

Fig. 2 Hansen solubility parameters of several solvents and the Hansen solubility sphere (elongated sphere) of the wax by-product at 25 °C. Solvents with circle markers fell within the solubility sphere, whereas solvents with cross markers fell outside the sphere

3.2 Preliminary solvent testing

The polarity of a solvent was a major contributor affecting the solubility of the lipophilic compounds in the tested solvent. Generally, lipophilic compounds are non-polar due to their size [14] and would therefore have increased solubility in non-polar solvents. Solubility of the lipophilic compounds in the chosen solvent decreased as the polarity of the solvent increased and increased with the addition of heat. For a tested solvent to be suitable for recrystallization, it was required that dissolution of the wax was dependent on the heating as to enable recrystallization when the mixture was cooled, and that the solvent was non-reactive with the various lipophilic compounds. A set of solvents with a range of polarities [15] were tested to evaluate the effect of solvent's polarity on its suitability for use in recrystallization. The method developed required the sample to be heated to 50 °C, at which most of the lipophilic molecules would have melted and freed the cannabinoid containing oil fraction. It was therefore critical that the solvents would not dissolve the waxes at temperatures lower than 25 °C since this would cause the waxes to partition with the solvent phase during the filtering stage.

Ethyl acetate had the second lowest polarity of the tested solvents with a RP of 0.228. This solvent performed according to all requirements, with only minimal dissolution of wax below 40 °C. After allowing to cool to 25 °C before filtering, most of the waxes recrystallized out of the solution forming a slurry with the solvent. Vacuum filtering was successful in separating the solvent phase from the waxes with minimal carry over of waxes to the solvent phase.

Ethanol (RP of 0.654) was a further good candidate solvent; however, the solvent started reacting with wax compounds at temperatures of 40 °C and higher and formed what was potentially an ester of a fatty acid present in the wax. Reaction of the solvent with components in the wax is highly unfavorable as the final goal of the extract is to be returned to the CBD extraction pathway, which undergoes various distillation stages downstream [3]. The introduction of a new product into the overhead CBD extraction pathway will negatively affect the downstream processing, and ethanol was therefore not a suitable solvent due to its reactivity.

Hexane (RP of 0.009), the solvent tested with the lowest polarity, dissolved all the wax material at room temperature when subjected to mixing. Minimal recrystallization of waxes took place when cooled to 25 °C and the majority of the lipophilic compounds partitioned into the solvent phase during filtering. Therefore, hexane was not suitable to be used as a solvent as minimal separation occurred between the waxes and the entrained cannabinoids at 25 °C and therefore did not meet selection criteria 2 and 4. Theoretically, by lowering the cooling point temperature, recrystallization can be improved. This will however increase processing costs as it will require refrigeration.

Acetone (RP of 0.355) was the last solvent tested for this method. As with ethanol, the acetone also reacted to form an insoluble product. When subjected to filtering with the by-product, the waxes did not effectively crystallize out of solution, resulting in significant carry over of waxes to the solvent phase. Therefore, acetone as a solvent performed the worst of the four for this method.

Ethyl acetate performed the best of all the tested solvents and therefore the limit on relative polarity was chosen as intervals around the RP of ethyl acetate. The limit was chosen as ± 55% of the RP of ethyl acetate (0.228), an arbitrary amount chosen as to not inadvertently exclude any solvents with potentially sufficient solvation ability, while still avoiding those which had been determined to not be suitable via experimentation.

3.3 Boiling point and safety of use

The first stage of the cannabinoid recovery procedure required heating of a wax by-product sample up to 50 °C to ensure that the lipophilic compounds melted and released the oils from the sample matrix. Therefore, a lower limit on normal boiling point (at atmospheric pressure) was set at 50 °C as all solvents with normal boiling points below 50 °C were not suitable to apply in this procedure. The upper limit on solvent boiling point was influenced by process considerations, specifically energy usage. After recovery of the cannabinoid-bearing solvent, the solvent would have to be removed through evaporation, to recover the cannabinoids for return to the overhead CBD isolation stream. Additionally, the temperature-induced decarboxylation of the cannabinoids is a further factor that needs to be considered in setting an upper limit on solvent boiling temperature. The decarboxylation of cannabinoids results in the conversion of the cannabinoid acids to the neutral form, e.g., CBDA to CBD. One study conducted on the kinetics of decarboxylation reported that there was significant loss of neutral cannabinoids when exposed to temperatures > 120 °C [16]. During distillation that occurs downstream of the wax removal in CBD isolation, it is assumed that decarboxylation takes place [3]; therefore, introduction of decarboxylated cannabinoids before the distillation stages may lead to degradation of the neutral cannabinoids. Therefore, the upper temperature limit needs to be below where decarboxylation would occur, and an upper limit of 105 °C was therefore implemented on the boiling point of potential solvents.

Limitations were further imposed on the relative polarity as a constraint on the solvation ability of a potential solvent. During the preliminary testing and the results from the HSP, it was seen that solvents with low RP values were able to dissolve the wax at 25 °C, which would prohibit the waxes from recrystallizing out of solution. This would ultimately result in the inability to separate the waxes from the cannabinoid containing solvent mixture through filtration. Therefore, the RP of a potential solvent had a lower limit to eliminate solvents that would have increased solvation ability of the waxes at 25 °C. An upper limit was further added to the RP of a potential solvent as solubility of the waxes at 50 °C was required to liberate the entrained cannabinoids.

The first exclusion of non-viable solvent options was therefore done based off the RP and T_b of a solvent. The limits implemented were as follows:

- For RP—only solvents having a RP of between 0.103 and 0.353 (± 55% of RP_{EtAce})
- For Tb—only solvents having a T_b of between 50 and 105 °C

With these constraints for the crystallization system, the initial list of 73 solvents was reduced to 13 solvent candidates that could be used for the recrystallization method. These solvents are depicted in the blue box as shown in Fig. 3.

To further narrow down the list of suitable solvents, the operational and health risks associated with each solvent were analyzed. The material safety data sheets (MSDS) of potential solvents were consulted in order to identify hazardous solvents. Solvents that were suspected of being carcinogenic (risk toward consumers) or were toxic (risk toward operators) were deemed unsuitable for the proposed recrystallization method. This step of evaluation removed an additional 6 potential solvents, as given in Table 3, leaving 7 solvents that would be suitable for the recrystallization method from the initial list of 73 proposed solvents. The 7 solvents that remained were 1,1-dichloroethane, 2-butanone, 3-pentanone, 1,2-dimethoxyethane, ethyl acetate, methyl acetate, and methyl t-butyl ether (MTBE). Solvents highlighted in red in Table 3 are deemed unsafe due to severity of associated risks.

3.4 Reactivity predictions via Kamlet-Taft parameters

In an attempt to predict reactivity of different solvents with the wax (as seen with ethanol and acetone), the Kamlet-Taft parameters for acidity, basicity, and polarity/polarizability (α, β, and π^*) were evaluated. The acidity (α) of a solvent quantifies hydrogen-bond donating ability and the basicity (β) quantifies hydrogen-bond accepting ability. Both these parameters are indicative of potential reactivity of a specific solvent in different circumstances.

The Kamlet-Taft parameters showed that all the solvents deemed suitable in Table 4 had similar acidity, basicity, and polarity. During preliminary testing, both testing with ethanol and acetone resulted in the generation of an insoluble, unknown product. When compared to ethyl acetate, it was seen that the acidity of ethanol was significantly higher (0.83 compared to 0), and the basicity was also higher (0.75 compared to an average of 0.48), as was the acidity of acetone (acidity of 0.08).

Based on the chemical reactivity and values for acidity for the solvents that were known to react with the wax, it was hypothesized that the acidity of a solvent was indicative of the reaction potential during the heating stage, and solvents

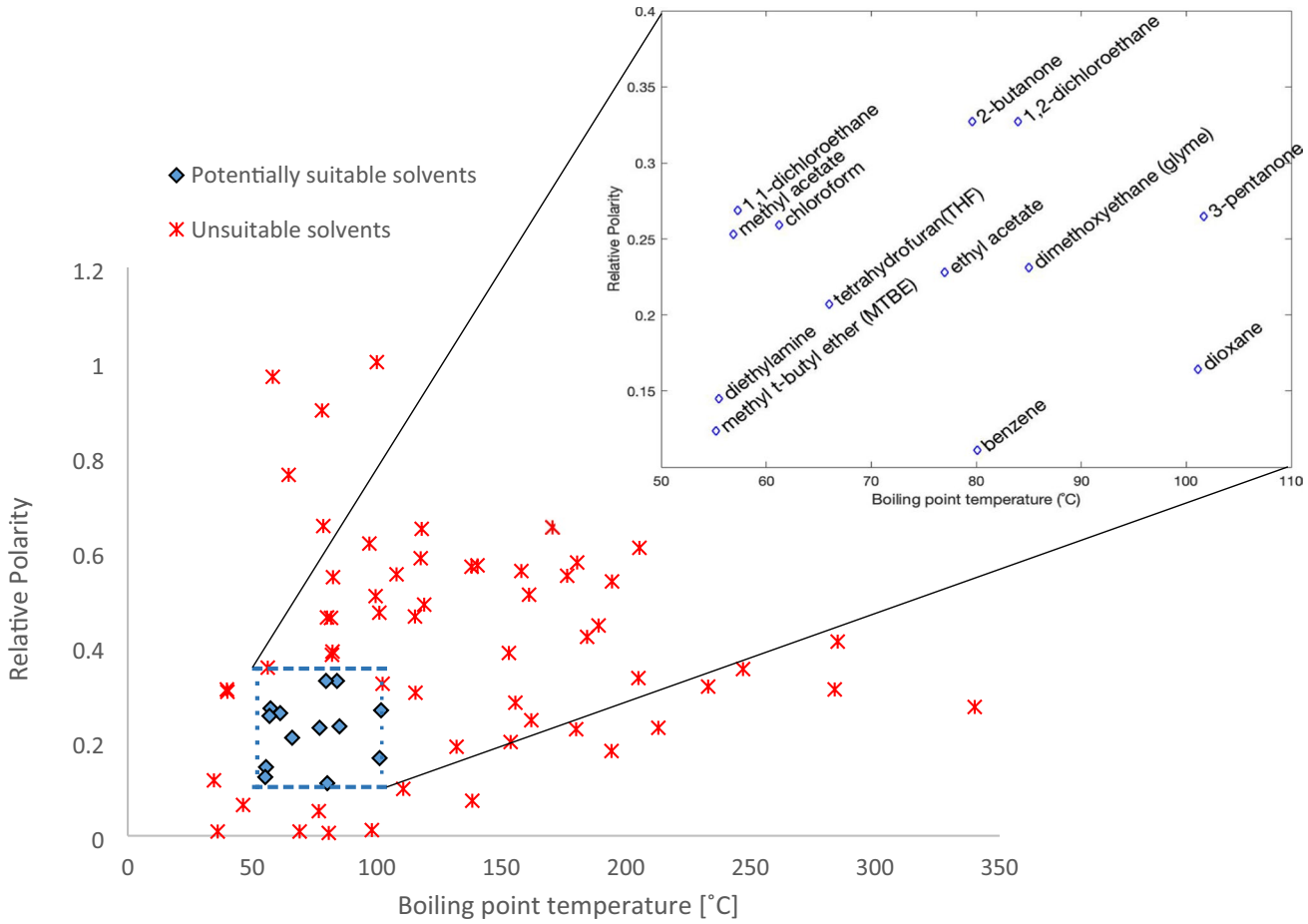

Fig. 3 Relative polarity of commonly used solvents versus their boiling point temperatures, with potentially suitable solvents displayed as diamonds and contained in a box, and unsuitable solvents displayed as a cross [15]. Names of solvents contained in the box are displayed in upper right corner

with an acidity other than 0 would most likely react. To validate this theory, 2-butanone that has an acidity of $\alpha = 0.06$ was tested for use in the recrystallization method and was also found to react. This led to the conclusion that it was not the class of solvent (alcohol, ketone, etc.) that was the main contributor to reactivity, but rather the hydrogen-bond donating ability. The exact nature of the reaction that took place warrants future investigation, specifically which fraction of the wax components is reacting, as this would influence the screening conditions when working on other plant wax substrates. For the scope of this study, the validation test done with 2-butanone justified the limitation placed on the Kamlet-Taft acidity of a potential solvent to be:

- All solvents with a Kamlet-Taft acidity not equal to 0 would be unsuitable for the proposed method

This final limitation set on the acidity of possible solvents decreased the list of potential solvents down to 5, which were 1,2-dimethoxyethane, 3-pentanone, ethyl acetate, methyl acetate, and MTBE. Testing of the identified solvents (all acidities = 0) resulted in non-reactive dissolution, therefore validating the imposed limitation on the Kamlet-Taft acidity.

The methodology developed in this study can be adapted for the liberation of entrapped oil fractions in other plant waxes as well, which often exploit differential solubility as a mechanism for separation [19]. The limitations imposed in the selection methodology developed by this study were governed by the requirements of recrystallization for the recovery of cannabinoids. However, the parameters evaluated would be applicable for fractionation of other plant waxes and the limitations on each can be adapted to fit the requirements of another fractionation methodology.

3.5 Experimental validation

The aim of the recrystallization technology was to recover entrained cannabinoids from the wax by-product generated

Table 3 Safety evaluation of proposed solvents with number of risk factors as reported in the respective MSDS. Solvents with too many associated risks are highlighted in grey and deemed unsafe

	Solvent name	1	2	3	4
1	1,1-dichloroethane	Flammable	Irritant		
2	**1,2-dichloroethane**	**Flammable**	**Irritant**	**Carcinogenic**	
3	2-butanone	Flammable	Irritant		
4	3-pentanone	Flammable	Irritant		
5	**benzene**	**Flammable**	**Irritant**	**Carcinogenic**	**Toxic**
6	**chloroform**	**Flammable**	**Irritant**	**Toxic**	
7	**diethylamine**	**Flammable**	**Irritant**	**Corrosive**	**Toxic**
8	1,2-dimethoxyethane	Flammable	Irritant		
9	**dioxane**	**Flammable**	**Irritant**	**Carcinogenic**	
10	ethyl acetate	Flammable	Irritant		
11	methyl acetate	Flammable	Irritant		
12	methyl t-butyl ether (MTBE)	Flammable	Irritant		
13	**tetrahydrofuran(THF)**	**Flammable**	**Irritant**	**Carcinogenic**	

Table 4 Kamlet-Taft parameters of the remaining potential solvents with water added as reference [17, 18]

Solvent	α	β	π^*
1,1-Dichloroethane	0.1	0.1	0.48
1,2-Dimethoxyethane	0	0.41	0.53
2-Butanone	0.06	0.48	0.67
3-Pentanone	0	0.45	0.72
Acetone	0.08	0.48	0.71
Ethanol	0.83	0.75	0.51
Ethyl acetate	0	0.45	0.55
Methyl acetate	0	0.42	0.6
Methyl t-butyl ether (MTBE)	0	-	-
Water	1.23	0.47	1.14

during commercial *Cannabis* processing, as these cannabinoids were disposed of along with the wax. To justify the limitations implemented on the various parameters, identified solvents were tested for use in the recovery of cannabinoids from the wax by-product through recrystallization. This involved using an identified test solvent to both dilute the wax during heating and also as a washing solvent during filtering. The ratios of solvent for dilution and washing to wax were tested at 3:1 and 2:1, respectively. The suitability of a solvent was evaluated based on dissolution of the wax at 50 °C, reactivity, recrystallization below 25 °C, and wax carry-over during filtration.

All five solvents as identified with the solvent selection methodology were tested as described above and were found to perform sufficiently for the recovery of cannabinoids through recrystallization. All solvents did not dissolve the wax at the lower temperature (25 °C), but did dissolve the waxes at the heating point temperature (50 °C). During cooling, the waxes did precipitate out of solution and were removed through filtration, with minimal carry-over of wax compounds to the cannabinoid extract. Carry-over of wax compounds to the extract was quantified to be less than 5% (w/w) of the extract by thermogravimetric analysis. No reactions took place during the experiments; therefore, validating that all criteria as set out for the recrystallization experiments were met through the solvent screening methodology.

4 Conclusions

This study is aimed at developing a robust solvent screening methodology to be used for fractionation of a *Cannabis* wax by-product through a novel recrystallization technology. During fractionation, it was aimed to recover entrapped

cannabinoids from the wax matrix with a secondary aim being to prepare the wax for further valorization processing. Preliminary solubility predictions were successfully done with the use of HSP, which showed that solvents with lower polarity would dissolve the wax at 25 °C. The selection methodology was done through limitations implemented on the polarity, boiling point temperature, and safety of use of potential solvents. Further refining of potential solvents was done through evaluation of the Kamlet-Taft parameters of solvents, which were found to be able to predict reactivity with the wax, when $\alpha \neq 0$. The developed methodology was tested on a set of commonly used solvents, which identified five suitable solvents out of 73. These five solvents were tested for cannabinoid recovery and were found to be suitable. The methodology developed in this study can be adjusted to be applicable for solvent selection in the fractionation of other plant waxes through adjustment of the selection criteria.

Abbreviations CBD: Cannabidiol; DMF: Dimethylformamide; DMSO: Dimethylsulfoxide; GC: Gas chromatography; HSP: Hansen solubility parameters; MS: Mass spectrometry; MSDS: Material safety data sheet; MTBE: Methyl tert butyl ether; Ra: Distance; RED: Relative energy difference; Ro: Radius of solubility sphere; RP: Relative polarity; Tb: Boiling point temperature; THF: Tetrahydrofuran; w: Weight.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval This declaration is not applicable to this manuscript.

Competing interests All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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