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PARD Engineering Turnkey Process Solutions **Date: 17.09.2024**

Extraction and purification of bioactive constituents from *Cannabis sativa* L.

Today, most of the USA, Canada, South America, Australia, and parts of Europe and Scandinavia have legalized cannabis for medicinal and, albeit less commonly, recreational use (**NASEM, 2017**). The use of psychoactive and pharmacologically active chemical compounds in cannabis leaves and inflorescence to treat chronic pain, nausea, spasticity related to multiple sclerosis, and epilepsy, among other conditions such as various cancers, have skyrocketed its popularity (**Blasco-Benito et al., 2018**; **Fathordoobady et al., 2019**; **Pratt et al., 2019**; **Rapin et al., 2021**) and led to the development of various methods for extraction, post-processing, and purification of cannabinoids, terpenes, and other compounds from cannabis biomass (**López-Olmos et al., 2022**; **Martinez et al., 2023**; **Qamar et al., 2021a**). With introduction of regulation 32661 on September 13th 2024 by the Ministry of Agriculture and Forestry, a legal basis for the widespread production of hemp for the extraction of bioactive compounds has been established (**T.C. Resmi Gazete, 2024**). Herein, we at PARD Engineering and Automation Co. Ltd. use our expertise in the fields of mechanical, mechatronic, and chemical engineering to offer prospective customers a complete solution for production of valuable products from cannabis using custom supercritical fluid extraction (SFE) systems.

Bioactive constituents of cannabis inflorescence

CBD and other cannabinoids

Over 177 terpenophenolic secondary metabolites known as cannabinoids have been identified in various Cannabis chemotypes (**Aizpurua-Olaizola et al., 2016**; **Hanuš & Hod, 2020**). Cannabinoids can account for as much as 30% of dry cannabis/hemp inflorescence (**Song et al., 2023**). Resin rich in cannabinoids is mainly produced in glandular tissue called trichromes, especially abundant in the buds of the female plant (**Potter, 2009**; **Zheljazkov & Maggi, 2021**) and illustrated in **Figure 1**. Tetrahydrocannabinol (THC) and cannabidiol (CBD) carry the highest significance due to the psychoactive properties of the former and the calming, antioxidative, cytotoxic, and pain-relieving pharmacological effects of the latter (**Yang et al., 2017**; **Rapin et al., 2021**). These and other cannabinoids are initially present in the plant tissue in their carboxylated form as tetrahydrocannabinolic (THCA), cannabidiolic (CBDA), and cannabichromenic (CBCA) acids (**Richins et al., 2018**). Cannabinoid acids undergo oxidative conversion to their neutral forms through

decarboxylation (**Olejar et al., 2021**). Neutral cannabinoids interact much more readily with neuro-receptors in the human body – CBD, for example, is >62 times more active than CBDA (**Song et al., 2023**).

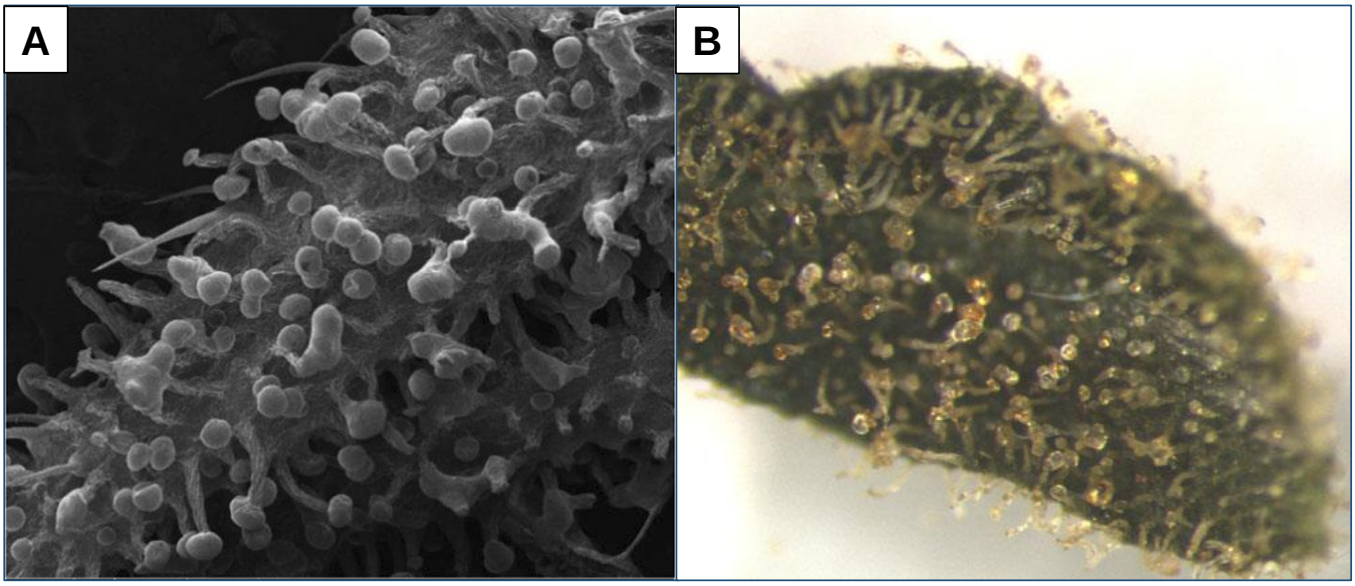

Figure 1: Close-up (A) and photo (B) of a floral bract with (non-)glandular trichromes (**Zheljazkov & Maggi, 2021**).

CBD is currently the most valuable non-psychoactive cannabinoid in hemp and is used in various grades of purity across many industries. The purest form, known as CBD isolate (>99% pure), is used by the pharmaceutical and cosmetic industries. Less pure distillates and CBD oils/extracts are dominant in the food, beverage, and supplement industries. Pure CBD commands a bulk price of 500–1000 USD kg^{-1}.

Terpenes and terpenoids

Hundreds of monoterpenes, sesquiterpenes and their terpenoids have been identified in cannabis and include a diverse range of hydrocarbon, alcohol, ester, ketone, aldehyde, and other functional groups that form the main essential oil fraction of the plant biomass (**Booth & Bohlmann, 2019; Hanuš & Hod, 2020; Sommano et al., 2020**) and impart strong anti-inflammatory and antioxidant properties (**Gallily et al., 2018**). Yields of cannabis essential oil vary widely between ca. 0.03–2.00 % of dry biomass (**Pieracci et al., 2021**; **Zheljazkov & Maggi, 2021**) and are rarely >0.25% of fresh biomass (**Bertoli et al., 2010**). Low yields relative to other aromatic plants contribute to a high price of ca. 1200 $ kg^{-1} (**Martinez et al., 2023**).

Terpenoid profiles are useful for identification and classification of cannabis chemotypes (a.k.a. chemovars). A simple comparison elucidates that chemovars of mostly *C. sativa* origin (producing more THC than CBD) are often higher in δ-3-carene, α-phellandrene, 1,8-cineole (eucalyptol), *cis*-β-ocimene, trans-β-ocimene, and terpinolene. In contrast, *C. indica*-dominant varieties (which produce mostly CBD) exhibit a

higher content of camphene, myrcene, and limonene (**Casano & Grassi, 2011**; **Hazekamp et al., 2016**). The average representative concentrations of the top 10 mono- and sesquiterpenes and terpenoids (by rate of occurrence) is illustrated in **Figure 2**.

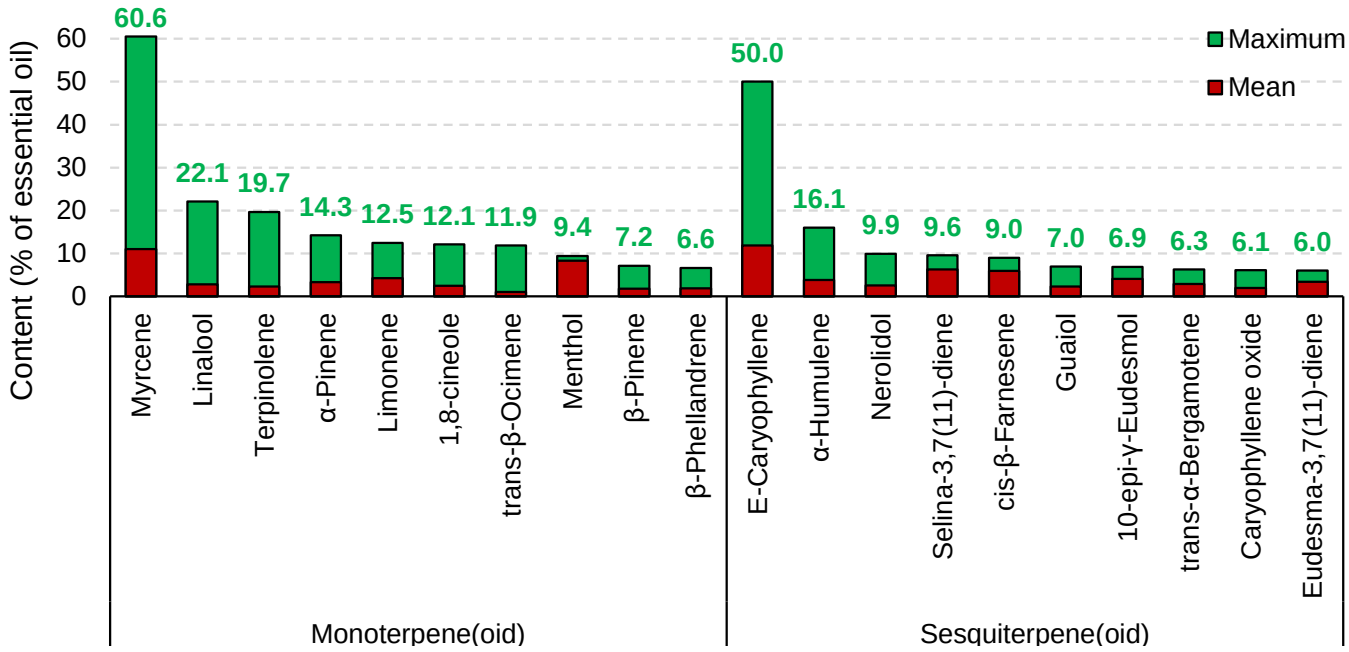

Figure 2: Maximum and mean content of top 10 mono- (A) and sesquiterpenes (B) in cannabis/hemp. Data from: Potter (2009), Bertoli et al. (2010), Casano & Grassi (2011), Hazekamp & Fischedick (2012), Da Porto et al. (2014), Hazekamp et al. (2016), Fischedick (2017), Naz et al. (2017), Blasco-Benito et al. (2018), Zheljazkov & Maggi (2021).

It is apparent that β-caryophyllene and myrcene are the most common (and often main sesqui- and monoterpene in cannabis), closely followed by α-pinene, β-pinene, α-humulene, and limonene (**Hanuš & Hod, 2020**).

Waxes

The cuticular waxes of cannabis are contained in the extracellular layer of leaves, flowers, and stems of the plant and are mainly comprised of hydrophobic lipids and other constituents. Alongside minerals, phospholipids, saccharides, and pigments, several *n*-alkanes are especially prevalent in the waxes (**Scognamiglio et al., 2022**), including pentacosane (C_{25}), heptacosane (C_{27}), nonacosane (C_{29}), and hentriacontane (C_{31}). Such components are usually undesired as they both dilute the concentration of volatile constituents and cannabinoids and have detrimental effects on the colour, clarity, consistency, and stability of the extract. Nonetheless, they are often co-extracted alongside terpenes and cannabinoids and are either removed in post-processing or as part of the extraction process.

Selecting an incorrect or unoptimized method for wax removal often leads to a significant loss of cannabinoids. Previous studies indicate that while the undesired *n*-alkanes and fatty acids comprised >13–28% and ca. 17% of the extract mass, respectively, cannabinoids were always the most abundant fraction at 39–50% of the precipitate, indicating a considerable loss of value (**Figure 3**).

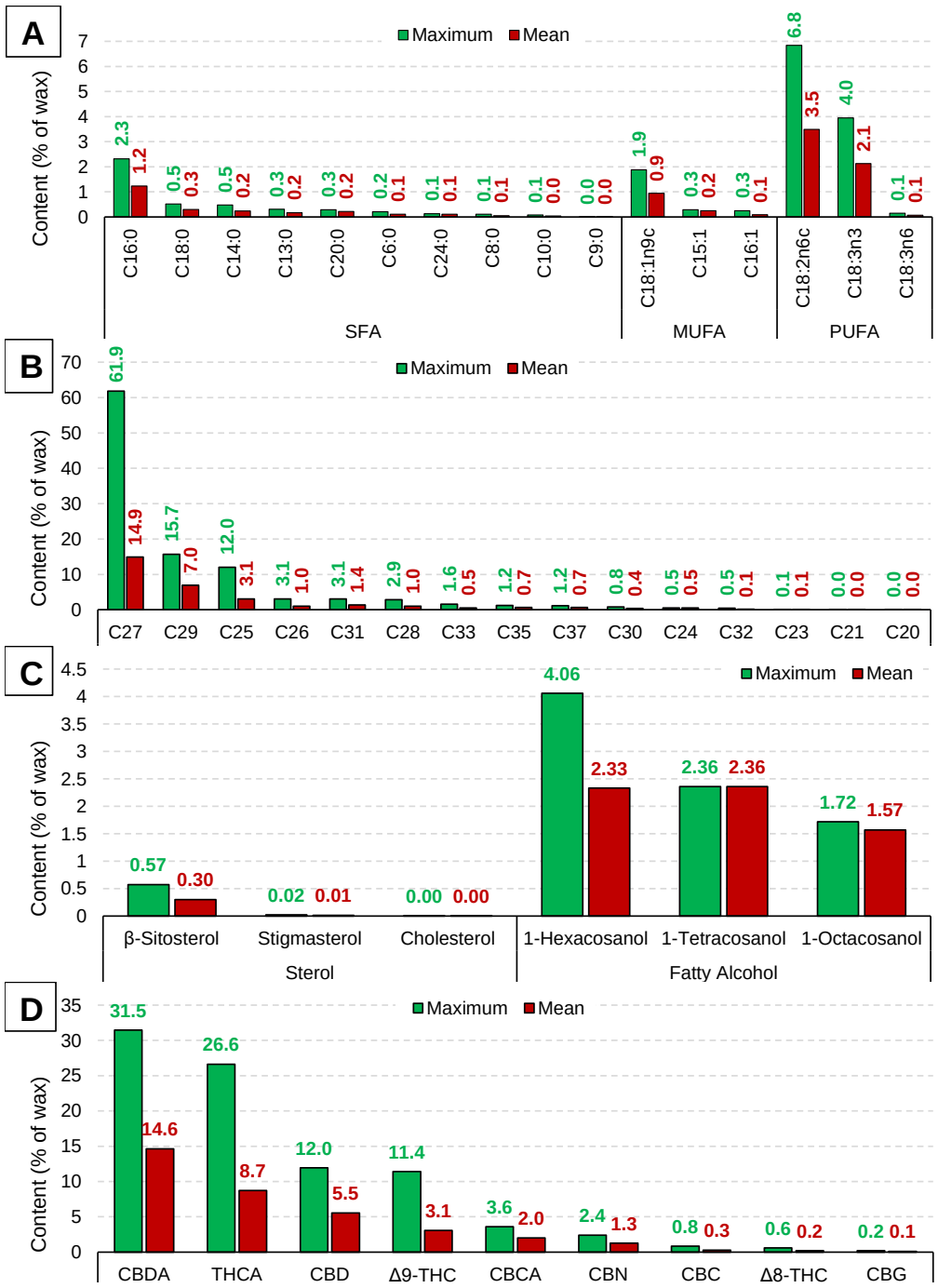

Figure 3: Content (%) of fatty acids (A), *n*-alkanes (B), sterols and fatty alcohols (C), and cannabinoids (D) in *C. sativa* wax after winterization. Data from: Leyva-Gutierrez et al. (2020), Scognamiglio et al. (2022), Duminy et al. (2023).

Extraction, fractionation, and purification of cannabinoids and terpenes

STEP 1: Storage and drying

Hemp inflorescence and leaves must be dried and milled for supercritical fluid extraction (SFE) to be effective. Common drying methods include slow-drying (i.e. air drying), dehumidifier-assisted drying, and the much more costly freeze drying, which reduces the temperature below the triple point of water under vacuum to induce sublimation and more effective breaking of trichromes (**Lopez-Olmos et al., 2022**). Hot-air drying is not recommended due to loss of volatile terpenes and terpenoids. Subsequent storage of the material is also important to avoid degradation. At room temperature, THC and other cannabinoids can decrease at a rate of 5% a month, while at -20 °C this impact is negated (**Martinez et al., 2023**).

STEP 2: Decarboxylation

Acidic cannabinoid forms present in inflorescence by default are polar and therefore significantly less soluble in supercritical CO_2 than their apolar neutral (phenolic) counterparts, such that decarboxylation prior to SFE favours extraction of more total cannabinoids. This may be carried out directly within the extraction vessel (**Olejar et al., 2021**; **Kargılı & Aytaç, 2022**) or in a dedicated convection or vacuum oven (**Moreno et al., 2020**) – at atmospheric pressure, the decarboxylation may not proceed to completion at temperatures <230 °C, though for all practical purposes the process substantially completes in 10–60 min at 100–150°C (**Song et al., 2023**). SFE efficiency and selectivity towards cannabinoids increases dramatically with decarboxylation pre-treatment, such that 10-fold concentration relative to content in plant material is achievable (**Marzorati et al., 2020**).

STEP 3: Supercritical Fluid Extraction (SFE)

SFE uses CO_2 in its supercritical state via their pressurization and heating past the critical point. Carbon dioxide (CO_2) is by far the most popular solvent (>90% of extractions; **Yousefi et al., 2019**) due to its abundance, low cost, environmentally benign (i.e. “green”) and non-toxic (GRAS status) nature, and a relatively low critical temperature (ca. 30.98 °C) and pressure (ca. 73.77 bar) that facilitates thermolabile compound extraction (**Span & Wagner, 1996**; **Anwar & Carroll, 2016**; **Sánchez-Camargo et al., 2019**). Additionally, the adjustability of CO_2 density and (by extension) solvating power by changing temperature, pressure, and/or other operating parameters allows for more selective extraction of natural materials and automatic CO_2 removal from extracted material by de-pressurisation.

SFE exhibits several advantages over conventional extraction techniques. The unique ability to control the solubility of analytes in $scCO_2$ by altering process parameters is a major advantage for process design allowing selective extraction and concentration of certain constituents. For example, **Kargılı & Aytaç (2022)** managed to produce a ca. 90% pure THC extract from a single $scCO_2$ extraction simply by optimizing the raw material (growth region and strain) and varying process conditions. The reduced temperature required for SFE is another benefit. Thus, mono- and sesquiterpene yields are often considerably higher in SFE relative to steam and hydrodistillation, and the extraction consumes less energy by a factor of ca. 2. Yields of approximately 10% dry inflorescence with >50% CBD content are expected from SFE.

STEP 4: Winterization

A high amount of unwanted cuticular waxes are co-extracted alongside desirable cannabinoids and volatile constituents. The first step following either extraction is therefore the removal of waxes and other organic-soluble lipids by a process termed “winterization”, which involves suspending and completely dissolving a crude extract in an organic solvent – usually ethanol at ca. 40°C and a solvent:crude extract ratio of 10:1 (v/v) – followed by significant cooling to -20°C or less. Commercial systems usually cool the solution to -40°C. Waxes subsequently precipitate into a heavy layer (**Figure 4**) due to reduced solubility in cold ethanol and the suspension is then filtered.

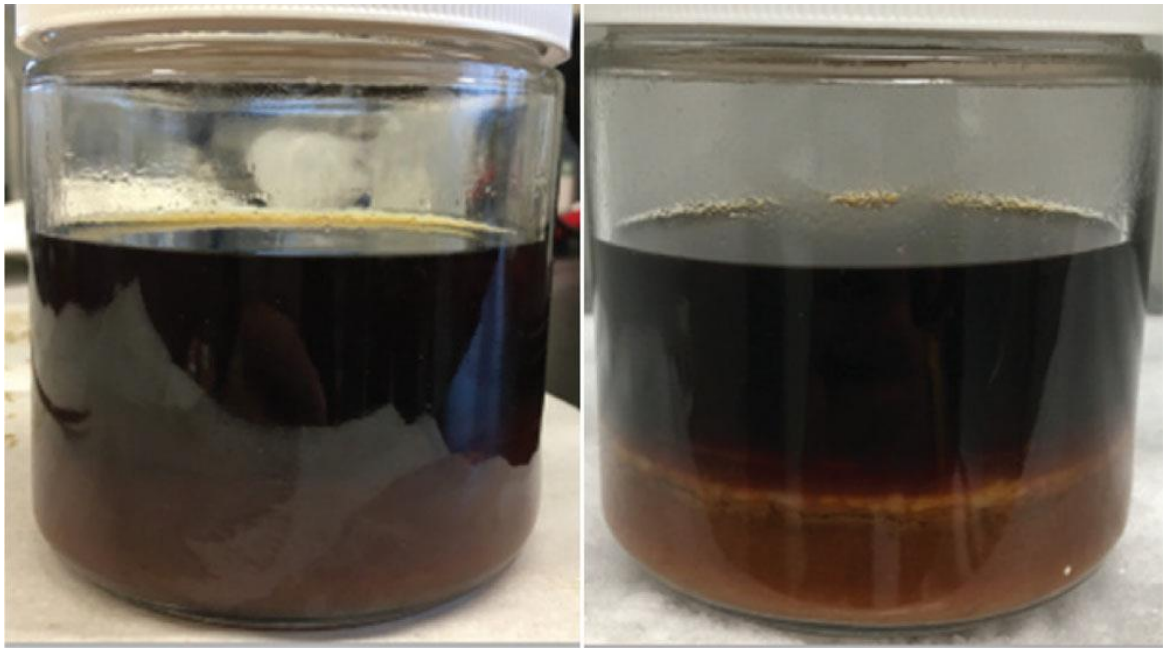

Figure 4: EtOH *C. sativa* extract before and after winterization (-80 °C). Pigments are still soluble in alcohol and remain in solution, necessitating colour remediation (Song et al., 2023).

Classical winterization requires a lot of chilling time and involves costly solvent recovery and residue testing. A key benefit of SFE is the ability to quickly and continuously remove co-extracted waxes from desirable terpenes and cannabinoids in a process termed “inline winterization” utilizing the fundamentally different solubility curve of the former. Terpenes, terpenoids, and cannabinoids are highly soluble in dense/liquid CO_2 even at low and sub-zero temperatures, while *n*-alkanes exhibit a solubility maximum at around 40 °C (at 90 bar) and experience a sharp decrease in solubility at lower temperatures such that their solubility becomes near-zero at temperatures of -10 °C to 0 °C. Since this observation was made by **Stahl and colleagues (1983)**, several authors have successfully applied this principle by isobarically cooling the highest-pressure separator to selectively precipitate waxes before dropping the pressure and collecting the rest of the extract. An example of the cannabinoid-rich fraction and precipitated waxes is shown in **Figure 5**. A simplified flowchart of a system suitable for wax removal is illustrated in **Figure 6**.

Figure 5: Examples of wax-free cannabinoid SFE extract (left) and waxes (middle, right) separated by inline winterization.

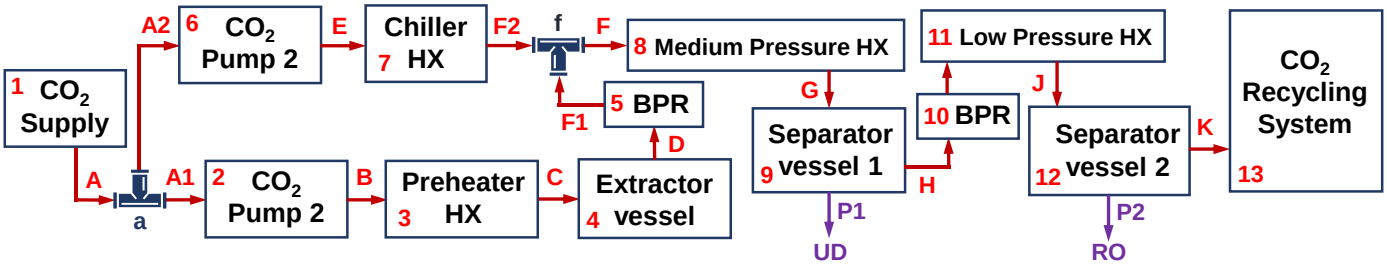

Figure 6: Simplified schematic of an SFE system with integrated wax removal/winterization.

STEP 5: Molecular distillation

Distillation is a secondary processing technique most often utilized for initial separation of terpenes and cannabinoids from remaining unwanted constituents (carbohydrates, pigments, phospholipids) in winterized, sub-micron filtered, and (optionally) colour-remediated extracts (**Song et al., 2023**). During

distillation, these components are sequentially vapourised through continuous boiling with subsequent condensation and collection. Thus, the process takes advantage of the differences in volatility, viscosity, vapour pressure and, therefore, the boiling point ranges to achieve effective separation. However, like most naturally occurring compounds, high-value constituents in *C. sativa* undergo thermal decomposition at high temperatures. To minimize this, distillation of cannabis and hemp extracts is universally carried out under vacuum to increase the volatility and vapour pressure of terpenes and cannabinoids, decreasing their boiling point and preserving composition.

The most scalable and effective distillation system is the Wiped Film Molecular Distillation (WFMD). The condensing and heating surfaces are housed within one chamber in WFMD, which is also able to reach evaporation surface areas of 45 m^2, feed rates of >1000 kg hr^{-1} (usually 4.5–18 kg hr^1 per m^{-2} of evaporator surface area), and pressures <1 Pa. In WFMD, an internal and is surrounded by the evaporator – usually a long-form jacketed (insulated) cylindrical chamber heated either electrically or via recirculating hot oil and including a rotating triple-bladed wiper (**Idárraga-Vélez et al., 2023**). The crude extract feed is pre-heated in the jacketed feed flask (e.g. to 100–115 °C), the metering valve is opened, and the material is gravity-fed into the distillation chamber. The wiper spins at an adjustable rate (e.g. 360–400 rpm for *C. sativa* extract and other viscous fluids) and spreads the feed material across the heated evaporator wall in a homogeneous thin film approximately 0.1–0.5 mm thick (**Song et al., 2023**). Diagonal inclination of the wiper blades coupled with a fast rotation speed constantly renew the thin film of feed material and shorten the exposure time to the heated environment. High vacuum (down to <1 Pa) facilitates quick vaporization of volatile constituents, which travel the short path to the condenser and condense back into a liquid – for viscous substances like cannabinoids it is recommended to circulate heated oil through the condenser at ca. 70–75 °C to prevent the formation of solid deposits on an excessively cool surface (**Sagili et al., 2023**). The condensed distillate and remaining less volatile material (term “residue”) are thus deposited into separate collection flasks. Any remaining vapours are trapped by the external condenser and cold trap before reaching the vacuum pump. The chamber has to be constructed such that intermolecular collisions along the travel path of the vaporised molecules toward the condenser are minimized, ensuring quick mass transfer and minimal degradation (**Idárraga-Vélez et al., 2023**; **Sagili et al., 2023**). A schematic representation of a single-pass WFMD system is shown in **Figure 7**.

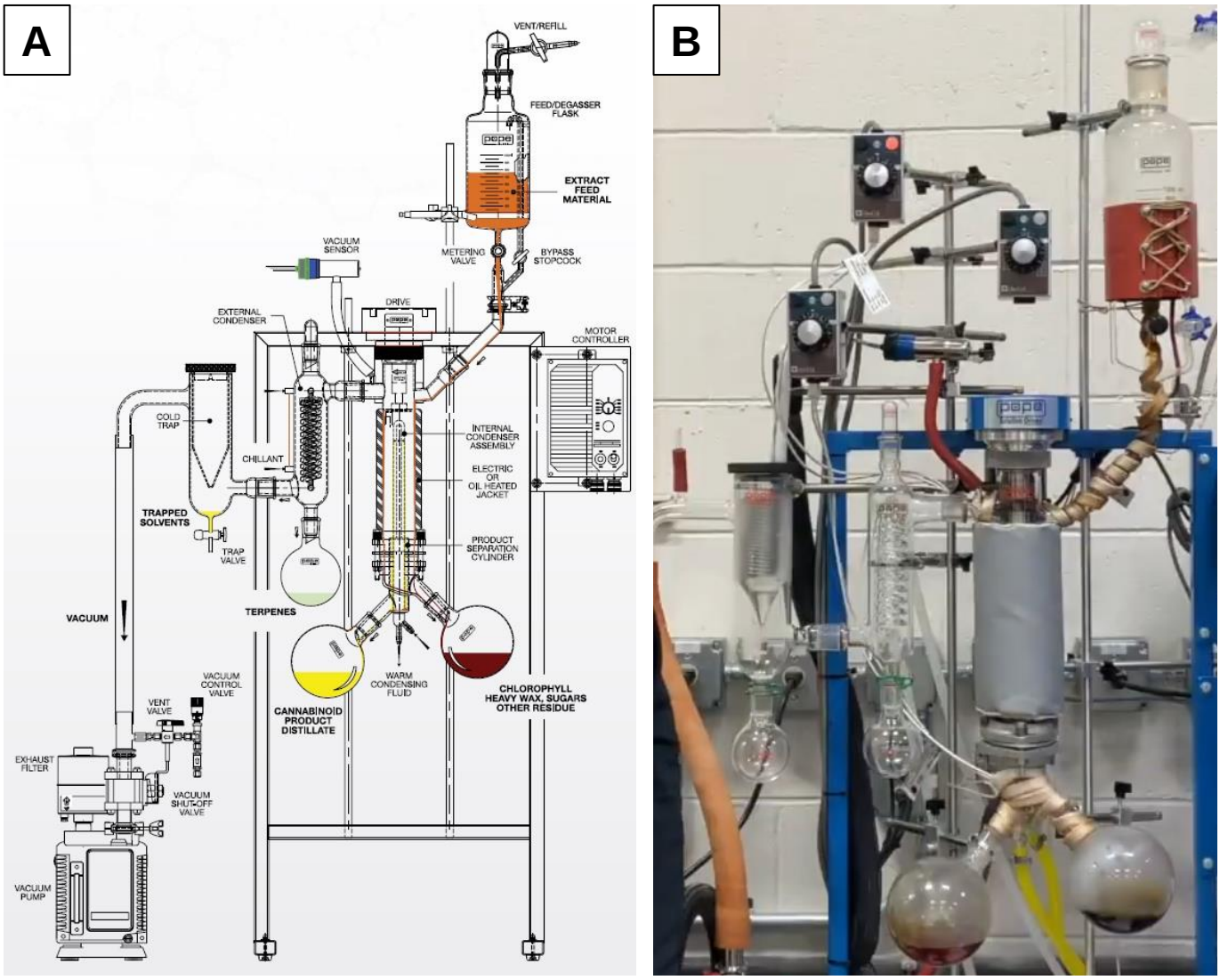

Figure 7: Scheme (A) and photograph (B) of a WFMD system showing the feed flask, distillation chamber, collection flasks, external condenser, cold trap, and vacuum pump.

The physical and vapour-liquid properties of *C. sativa* extract constituents necessitate a two-pass distillation process, where the first pass strips the cannabinoids from the feed by separating terpenes into the distillate (**Valizadehderakhshan et al., 2022**; **Sagili et al., 2023**). The second pass distils the cannabinoids, leaving any remaining waxes, sugars, and other heavier molecules in the residue. However, with SFE as the extraction technique, terpenes can be extracted with subcritical or supercritical CO_2 such that only a single-pass WFMD is subsequently required. A CBD distillated is expected to be >75% pure, with >80% of CBD present in the original crude extract recovered.

STEP 6: Crystallisation

Cooling crystallisation is often the final processing step applied to a concentrated CBD-rich cannabinoid distillate to isolate and purify the CBD to >99% in a single processing step. The solubility of CBD and most other organic molecules increases with solution temperature and decreases with concentration. Once the (metastable) crystallisation temperature is reached, the system enters the metastable supersaturation state and nucleation proceeds either through formation of new crystals from a clear solution (primary nucleation) or incremental growth of existing crystals seeded into the solution (secondary nucleation). Initial crystallisation temperature gradually decreases throughout the course of crystal growth due to the associated decrease in solution concentration, necessitating continued cooling (**ter Horst et al., 2015**). Substantial or complete insolubility of the target solute at practically achievable temperatures is required for successful crystallisation. Determining the cloud point (crystallisation) and clear point (dissolution) temperatures at various solute concentrations allows determination of polythermal solubility throughout the feasible range of process conditions (**Figure 8**).

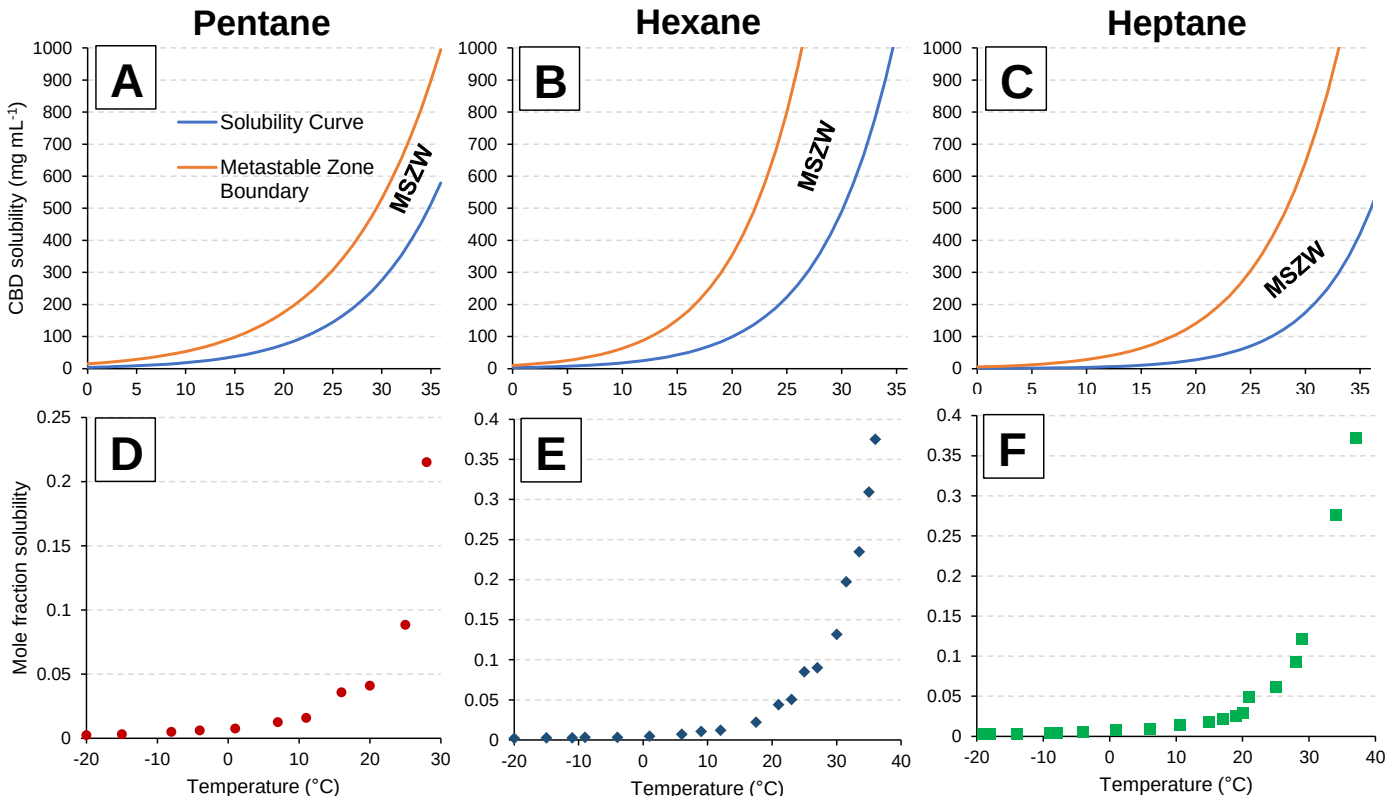

Figure 8: CBD polythermal solubility data in *n*-pentane, *n*-hexane, and *n*-heptane: (A–C) Concentration (mg mL^{-1}) versus temperature showing the MSZW (Technobis, 2023). Note the wider MSZW for heptane; (D–F) Molar solubility fractions at different temperatures (Pulido Bonilla et al., 2023) – note the limited temperature range for *n*-pentane.

The cloud points and clear points are plotted into the supersolubility and solubility curves, respectively. Above the metastable zone boundary (supersolubility curve), primary nucleation results in rapid precipitation of crystals through primary nucleation. Dissolution occurs below the solubility curve. The range of conditions between the two curves is known as the metastable zone width (MSZW), where spontaneous primary nucleation will not occur, but secondary nucleation will proceed at a predictable rate. A wider MSZW therefore allows more control over crystal growth by secondary nucleation (**Technobis, 2023**). For CBD crystallisation, hydrocarbon solvents like *n*-pentane, *n*-hexane, or *n*-heptane are preferred.

CBD crystallisation is carried out in a jacketed and insulated glass or stainless steel reactor (20–100 L; **Figure 9**) connected to a temperature control unit and equipped with a Teflon stirrer and a sintered filtration plate with a drain valve underneath the chamber. A substantially pure distillate (>70% CBD) free of waxes yields >99% pure CBD on first crystallisation at a 70–80% yield. The crystals are finally dried under vacuum using various methods.

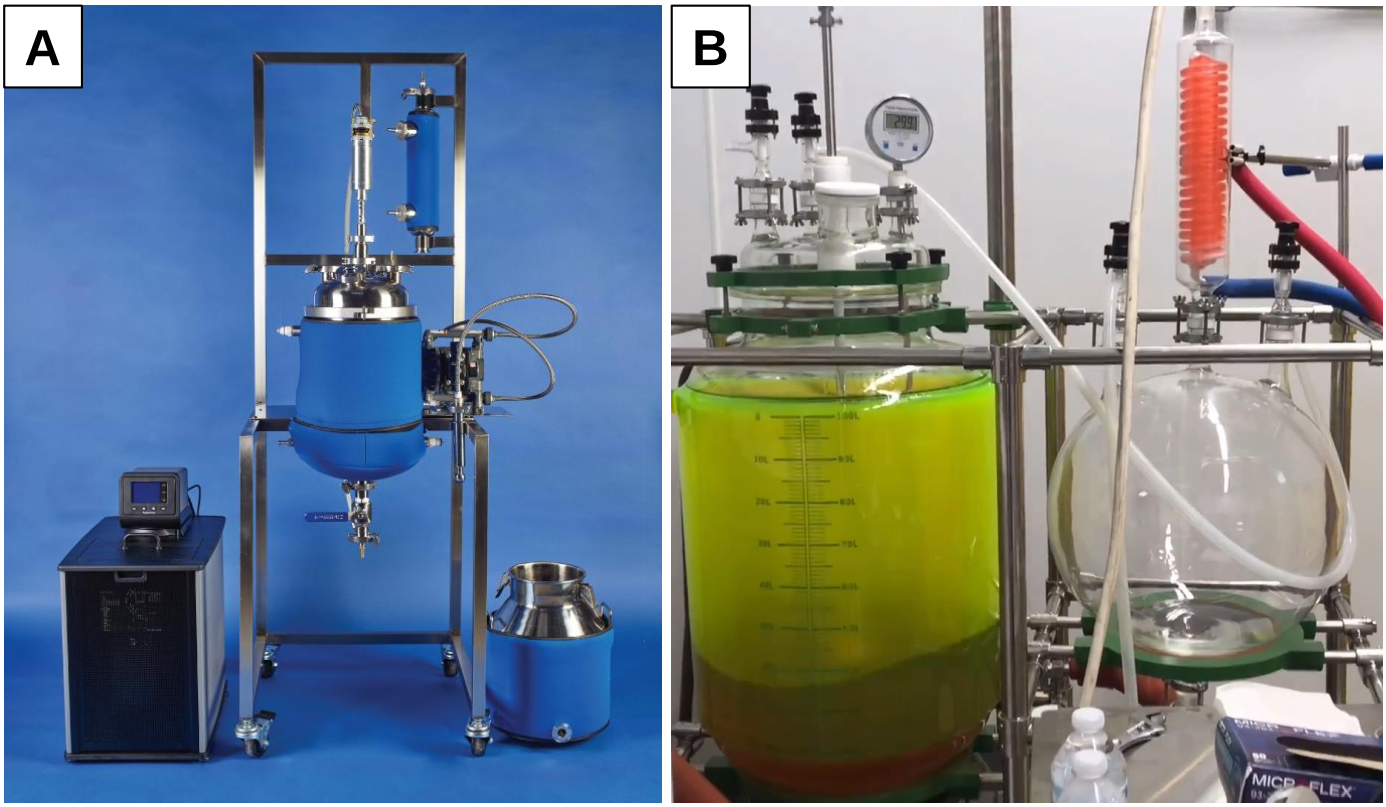

Figure 9: Examples of stainless steel (A) and glass (B) CBD crystallisation reactors. Note the green semi-transparent jacket and separate vacuum flask with condenser for safe retrieval of the mother liquor.

Our Offer

At PARD Engineering and Automation, we have multiple decades of combined experience producing custom high-pressure systems for subcritical and supercritical fluid extraction of natural products. Moreover, the chemical engineering expertise of our R&D partners allows us to configure and manufacture not only the required machinery, but engineer and optimize the extraction process parameters, assess Cost of Manufacturing, and provide analytical laboratory services. Thus, we are able to offer truly turnkey process solutions to prospective customers. Provided with a suitable raw material, our **hemp extraction process** includes the following components with guaranteed performance outcomes for the customer:

1) Complete optimization and setup of required machinery for material drying. Methods include a moisture-controlled cabin and/or freeze drying.

2) Setup of a decarboxylation process, including reactor design, manufacture, and process optimization of temperature, pressure, and heating duration.

3) Design, manufacturing, process optimization, and long-term technical service of a scalable supercritical CO_2 extraction system with inline winterization technology to obtain a wax-free crude extract. Optimized parameters include extraction pressure and temperature programs, flow rate, winterization parameters. A pilot-scale (20+20 L) SFE system manufactured by PARD Engineering in-house is pictured in **Figure 10**.

Figure 10: A pilot-scale SFE extraction system equipped with inline winterization.

4) Configuration, procurement, and process optimization of a molecular distillation system to obtain CBD distillate and terpene fractions. Optimized parameters include process pressure, temperature program, feed solution flow rate, cold trap configuration, and other parameters.
5) Configuration, procurement, and process optimization of a cooling crystallisation reactor.
6) If required, we also optimize the extraction and colour remediation of hemp seed oil rich in tocopherols (vitamin E) and ω-3 linolenic acid. The seed residue may also be used as a significant and rich source of essential amino acids and crude protein.

All of the above services will be supported by our state-of-the-art chromatographic laboratory equipped with both liquid and gas chromatograph and staffed with PhD-educated analytical chemists and chemical engineers. We provide full output data for all processes. A non-exhaustive subset of process performance metrics routinely achieved is listed in **Table 1**.

Table 1: Performance metrics typically achieved by PARD Engineering turnkey cannabis extraction.

Process step	Performance metric	Target value(s)
Pre-treatment	Dry material moisture content	<13%
	Decarboxylation efficiency (CBDA → CBD conversion)	>95%
Supercritical CO_2 Extraction + **inline winterization**	SFE extraction yield	>15% dry weight
	CBD content	>50%
	Total THC content	<0.3%
	Total cuticular wax content	≤0.1%
Molecular Distillation	CBD content of terpene fraction	<15%
	CBD content of cannabinoid fraction	>75%
	CBD recovery from crude extract	>90%
	Total THC content	<0.3%
Cooling Crystallisation	CBD recovery from distillate	>80%
	Final crystallised CBD purity	>99%
	Total THC content	<0.3%

A flowchart of the complete extraction, fractionation, and purification process is shown in **Figure 10**.

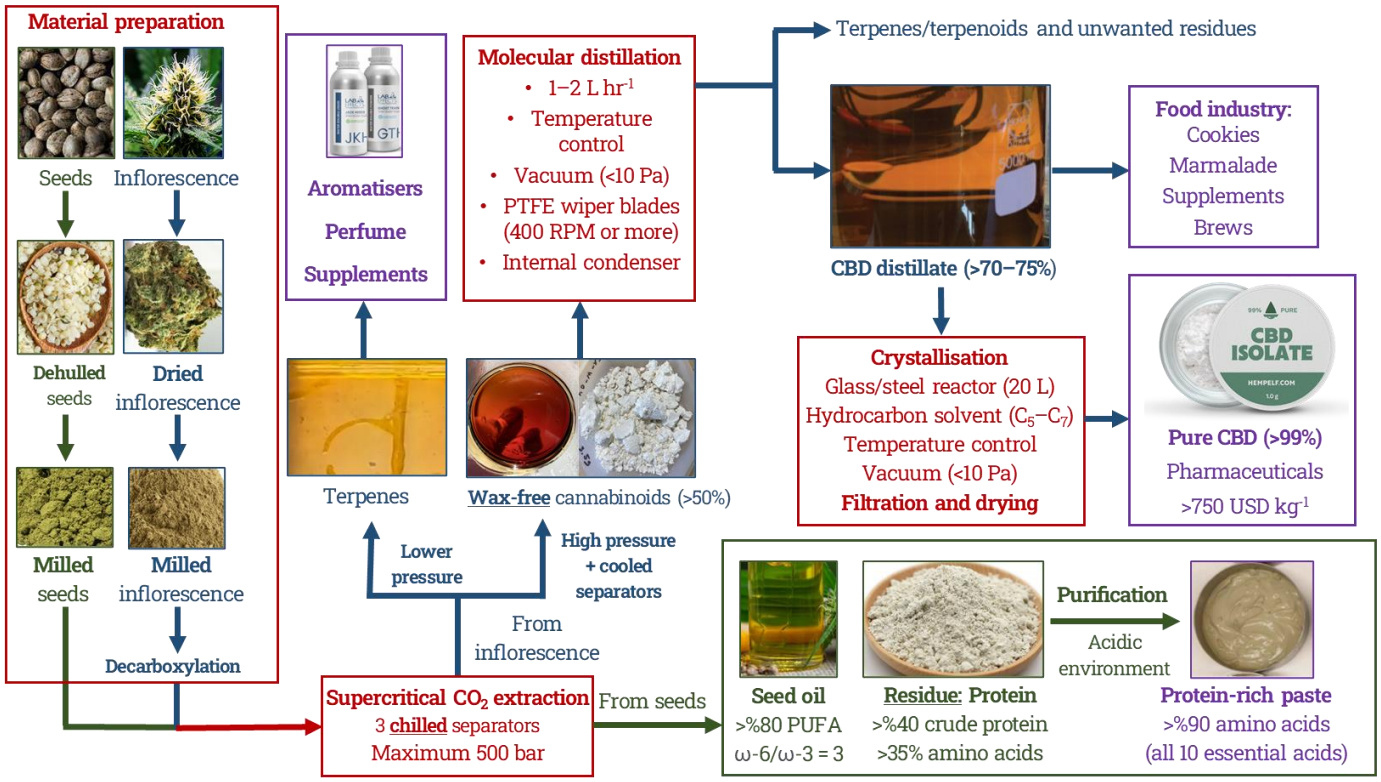

Figure 10: A simplified representation of turnkey hemp extraction processes offered by PARD Engineering.

Process example – 30 L capacity SFE system

Here we present an example processing capacity evaluation to obtain pure CBD from fresh hemp inflorescences supplied with a 30 L SFE system. Given a typical dry cannabis density of 250 g/L, a 30 L system will accommodate 7.5 kg of inflorescences per batch. Assuming 24-hour operation, approximately 5 batches can be processed daily (total of 37.5 kg per day). Since convection oven drying and decarboxylation at a constant temperature rise rate from 40 °C takes approximately 10 hours, a convection oven with a capacity of 40 kg (or at least 250 L) per batch is required. The dry material is then loaded into the SFE system for extraction. From 37.5kg, expect at least 5.6kg of crude extract per day containing >50% CBD. Distillation and purification equipment suitable for such a setup remains on a low-capacity pilot scale and can be installed and optimized relatively easily. A pure CBD yield of approximately 70% (2.8 kg to 2.0 kg) can be expected daily from the starting content in the crude extract. Supplements include terpene-rich essential oil (up to 600 mL daily). In addition to moving the entire crude extract to the pure CBD stage, intermediate products such as CBD oil (>60% wax-free CBD), CBD distillate (>80%) or full spectrum oil (wax-free crude extract) can also be produced and sold.

Building on this capability, we offer a complete turnkey solution for CBD-rich hemp processing. Our offer includes the design and manufacture of SFE equipment, supply and installation of all ancillary equipment, process optimization for pre-treatment, extraction and purification of hemp extracts and all relevant laboratory analyzes performed in a state-of-the-art chromatographic laboratory. Our offer is modular. If, as our customer, you decide to purchase auxiliary equipment separately from our offer, we will provide all configuration details and assist with the installation.

The system includes a 30 + 30 L extraction capacity at 500 bar maximum working pressure, dual separators (10 L each) for online wax removal by winterization, a co-solvent pump for optional addition of ethanol into the CO_2 inflow. We can optionally configure and source auxiliary equipment required for the process, including a decarboxylation oven, cutters for material comminution, molecular distillation systems, crystallisation reactors, and all associated heater and chiller units as well as pumps. This system is scalable up to a working volume of 1000 L able to process an approximate 1250 kg of hemp and produce 108 kg of pure CBD daily (provided all auxiliary equipment).

We hope you find this offer interesting. Contact us today to discuss your turnkey extraction facility.

Yours sincerely,

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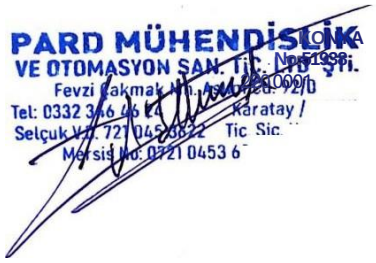

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