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Evaluation of Cannabinoid and Terpenoid Content: Cannabis Flower Compared to Supercritical CO_2 Concentrate

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ABSTRACT

A recent cannabis use survey revealed that 60% of cannabis users rely on smelling the flower to select their cannabis. Olfactory indicators in plants include volatile compounds, principally represented by the terpenoid fraction. Currently, medicinal- and adult-use cannabis is marketed in the United States with relatively little differentiation between products other than by a common name, association with a species type, and Δ-9 tetrahydrocannabinol/cannabidiol potency. Because of this practice, how terpenoid compositions may change during an extraction process is widely overlooked. Here we report on a comparative study of terpenoid and cannabinoid potencies of flower and supercritical fluid CO_2 (SC-CO_2) extract from six cannabis chemovars grown in Washington State. To enable this comparison, we employed a validated high-performance liquid chromatography/diode array detector methodology for quantification of seven cannabinoids and developed an internal gas chromatography-mass spectrometry method for quantification of 42 terpenes. The relative potencies of terpenoids and cannabinoids in flower versus concentrate were significantly different. Cannabinoid potency increased by factors of 3.2 for Δ-9 tetrahydrocannabinol and 4.0 for cannabidiol in concentrates compared to flower. Monoterpenes were lost in the extraction process; a ketone increased by 2.2; an ether by 2.7; monoterpene alcohols by 5.3, 7 and 9.4; and sesquiterpenes by 5.1, 4.2, 7.7, and 8.9. Our results demonstrate that the product of SC-CO_2 extraction may have a significantly different chemotypic fingerprint from that of cannabis flower. These results highlight the need for more complete characterization of cannabis and associated products, beyond cannabinoid content, in order to further understand health-related consequences of inhaling or ingesting concentrated forms.

Introduction

A recent survey reported that when users select *Cannabis sativa* L. (Cannabaceae) for medical purposes, 60% use the scent as part of their selection process. Inhalation of flower, either by smoking or vaporization, is still the most common administration method, but cannabis-derived herbal products, made by conventional solid-liquid extraction (concentrates), are also being utilized for inhalation and orally [1]. There is an increasing trend to produce highly concentrated cannabis products for both medical and adult use in the United States [2, 3]. For instance, regulation in the State of New York only allows sale of concentrated product, not flower [4]. In Canada, concentrates and flower are available, but concentrates are only for oral administration, not for inhalation or use in edible products [5].

Concentrates are typically highly viscous oils sold under the names “wax,” “shatter,” “vape oil,” or “crumble,” depending on the extraction process. These concentrates may be inhaled using

ABBREVIATIONS

CBD	cannabidiol
CBDA	cannabidiolic acid
CBG	cannabigerol
CBN	cannabinol
SC-CO_2	supercritical carbon dioxide
THCA	Δ-9 tetrahydrocannabinolic acid
THC	Δ-9 tetrahydrocannabinol

portable pens (electronic cigarettes or e-pens) or by a process known as "dabbing" [6]. Dabbing involves the flash vaporization of the concentrate by rapidly heating the "dab" to 300–400 °C (typically on a titanium rod) followed by inhalation of the vapor with a specialized pipe. This dose has been reported to be around 40 mg/dab (of THC), and is reported to induce negative side effects such as catatonia, paranoia, anxiety, psychosis, and listlessness [7].

SC-CO_2 has many applications in agricultural and botanical medicine industries. The application of this methodology has previously been shown to be amenable for extraction and separation of cannabinoids and terpenoids, as well as fatty acids in hemp seed oil [8, 9]. CO_2 is commonly used as a solvent for practical reasons including its lack of toxicity, affinity for a wide range of organic compounds, ease of disposal, and availability at low cost [10]. CO_2 can be easily separated and recovered from the extract and is expected to leave the finished product with a good reproduction of flavor and fragrance of the starting material [10]. For some of these reasons, the cannabis industry has rapidly adopted SC-CO_2 as a method of choice for both a retail product in itself (for vaporization or "dabbing") and other products such as foods, candies, or topicals, such as creams or balms.

While some consider that cannabinoids constitute the principal "active ingredient" in cannabis, over 100 terpenoids have been identified in cannabis species and as bioactive molecules contribute to the overall effects [11–14]. Terpenoids are a large class of plant secondary metabolites, derived from isoprene units, and are volatile organic compounds [15]. It is known that these metabolites can be modified by soil nutrients and plant stress such as insect attack or light and heat [16–18]. The essential oil component can significantly change simply upon drying of the flower material [13, 19].

It was reported that steam-extracted cannabis essential oil has a monoterpene content of 92% and sesquiterpene content of 7% [13]. These volatile compounds are not as well studied as cannabinoids; however, the essential oil fraction has been well documented and established in the literature [20–23]. Studies suggest that there are 17 predominant terpenoids common to the majority of strains and are considered with cannabinoids to be chemotaxonomic markers for cannabis [19, 24–26]. Some terpenoids are known to have narrow therapeutic windows when ingested, but less is known about any toxicity associated with chronic inhalation of concentrated terpenoids in cannabis. Paracelsus, a Swiss-German philosopher (1493–1541) now considered to be a father of modern medicine, is quoted as saying *"Sola dosis facit venenum"*, literally, "Only the dose makes the poison" [27]. There have been few reports on production protocols and chemical composition of cannabis products being sold and used under regulated U. S. cannabis markets. A report from the Netherlands compared extraction products using naphtha, petroleum ether, ethanol, and olive oil. This study showed differential extraction of terpenoids, yet it was unknown whether this was due to degradation, loss to evaporation, and interaction with solvent components or whether they were simply not extracted [28]. In their report, the monoterpenes myrcene and terpinolene and the bicyclic sesquiterpene *β*-caryophyllene were retained across all extractions. A recent attempt has been made to further optimize a SC-CO_2 extraction method with ethanol as a co-solvent and using focused ultrasound extraction to first "de-terpinate" the matrix prior to extraction of cannabinoids [29]. This method used a lower temperature and allowed for retention of the monoterpenes.

A recent survey of cannabis use reported that 61% of responders had ever "vaped," while only 12% report that it is their preferred method. The most popular device was an e-pen (for concentrate use); however, experienced users preferred vaporization devices for flower over concentrates [30]. Another survey of medical users reported that inhalation is the preferred delivery method of 84% of responders but 68% of these responders were smoking [1].

Because the flower and their respective concentrates are sold by the same common chemotype names (previously referred to as "strains"), this implies the consumer would be acquiring the same chemotypic profile. We directly compared the flower to the extraction product (referred to as "concentrate"), with the assumption that the flower trim (used in the SC-CO_2 extraction) can be considered representative of the flower. We report on six cannabis chemotypes and compare the ratios of two major cannabinoids and their 14 most common terpenoids between mature flower and its SC-CO_2 extraction correlate.

Results

Five of the chemotypes tested were known to be type 1 (THC-dominant) plants (CBD : THC < 0.5) and one to be a type 2 (CBD : THC 0.3–0.5) [31]. The type 1 flowers ranged 17–27% THC_{max} (mean = 24% ± 4). The THC potency for type 1 concentrate ranged 67–76% THC_{max} (mean = 73% ± 4.4) (▶ **Fig. 1 A**). There was no significant quantity of CBD_{max} in the type 1 flower (average of 0.2%) and in concentrate the average amount was 1.2%. The type 2 plant was 9% THC_{max} and 10% CBD_{max} compared to 35% and 41%, respectively, in the concentrate (▶ **Fig. 1 B**). This change is a 3.2-fold increase in THC potency for the type 1 and 4-fold increase in CBD potency for the type 2 flower compared to concentrate. There were no significant amounts of other cannabinoids detected in either product type (see ▶ **Table 1**).

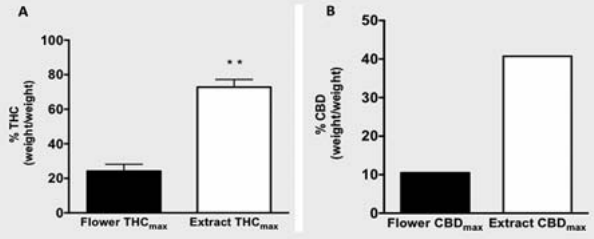
All of the chemovars had somewhat similar terpenoid profiles, with minor variations in ratio and composition (▶ **Fig. 2**). A summary of the aggregate average terpenoid fold-change between flower and concentrate is displayed in ▶ **Fig. 3**. Monoterpenes were not concentrated in any of the extracts, with flower having higher content of monoterpenes than the concentrate. *α*-pinene was significantly higher in two flower chemotypes ($p < 0.005$)

▶ **Table 1** Cannabinoid content of six Cannabis chemovars.

Chemovar	CBDA (mg/g)		CBD (mg/g)		THCA (mg/g)		THC (mg/g)		CBN (mg/g)		CBG (mg/g)	
	F	C	F	C	F	C	F	C	F	C	F	C
Cherry Kush	0.3 (± 0.06)	91.2 (± 2.1)	ND	5.3 (± 0.6)	264 (± 3.5)	693.8 (± 52)	1.6 (± 0.9)	77.5 (± 1.9)	ND	1.5 (± 0.06)	ND	ND
Blackberry Kush	0.5 (± 0.02)	7.0 (± 0.5)	ND	0.9 (± 0.6)	267.4 (± 2.8)	749 (± 84)	40.9 (± 1.2)	105.6 (± 2.3)	1.0 (± 0.0)	11 (± 0.6)	2.0 (± 0.0)	7.9 (± 0.6)
Pineapple Kush	0.5 (± 0.03)	3.3 (± 0.6)	ND	1.9 (± 0.5)	235.6 (± 2.6)	650.5 (± 45)	24.3 (± 1.5)	174.3 (± 3.8)	ND	1 (± 0.2)	ND	1.1 (± 0.2)
Purple Sour Diesel	0.5 (± 0.02)	20.3 (± 6.0)	ND	ND	24.24 (± 3.1)	674.9 (± 44)	19.4 (± 1.0)	9.98 (± 2.7)	0.1 (± 0.01)	15 (± 0.07)	1.3 (± 0.0)	20.5 (± 0.9)
Ripped Bubba	0.4 (± 0.02)	3.1 (± 0.2)	ND	ND	24.58 (± 4.2)	714.7 (± 53)	ND	9.68 (± 2.1)	ND	1.6 (± 0.7)	ND	11.4 (± 0.8)
Harlequin	117.5 (± 2.1)	444.6 (± 3.7)	1.7 (± 0.6)	17.4 (± 0.9)	88 (± 1.4)	333 (± 26)	9.3 (± 2.2)	55.0 (± 1.5)	ND	ND	0.7 (± 0.03)	ND

Data are expressed as the mean in mg/g with standard deviation in parenthesis. Each sample was run in triplicate. (ND is not detected or below the limit of quantification.) F: flower; C: concentrate.

and compared to concentrates there was a loss of α-pinene and β-pinene in the extraction process by factors of 0.7 and 0.8, respectively. β-myrcene was significantly concentrated in only two concentrates and lost in two others. Limonene was significantly higher in two flowers versus concentrate and did not have a positive fold-change (0.6 concentration factor). Linalool was significantly higher in five concentrates by a factor of 5.3. The bicyclic sesquiterpene β-caryophyllene was significantly increased over flower in the five concentrates, by a factor of 5.1. Terpene alcohols were increased by 5.3-, 7-, and 9.4-fold across the aggregate, and other sesquiterpenes by factors of 5.1, 4.2, 7.7, and 8.9. (Only three of the samples contained α-bisabolol and guaiol.) Additional details of the quantification in each chemovar can be found in ▶ **Table 2** and **3**.



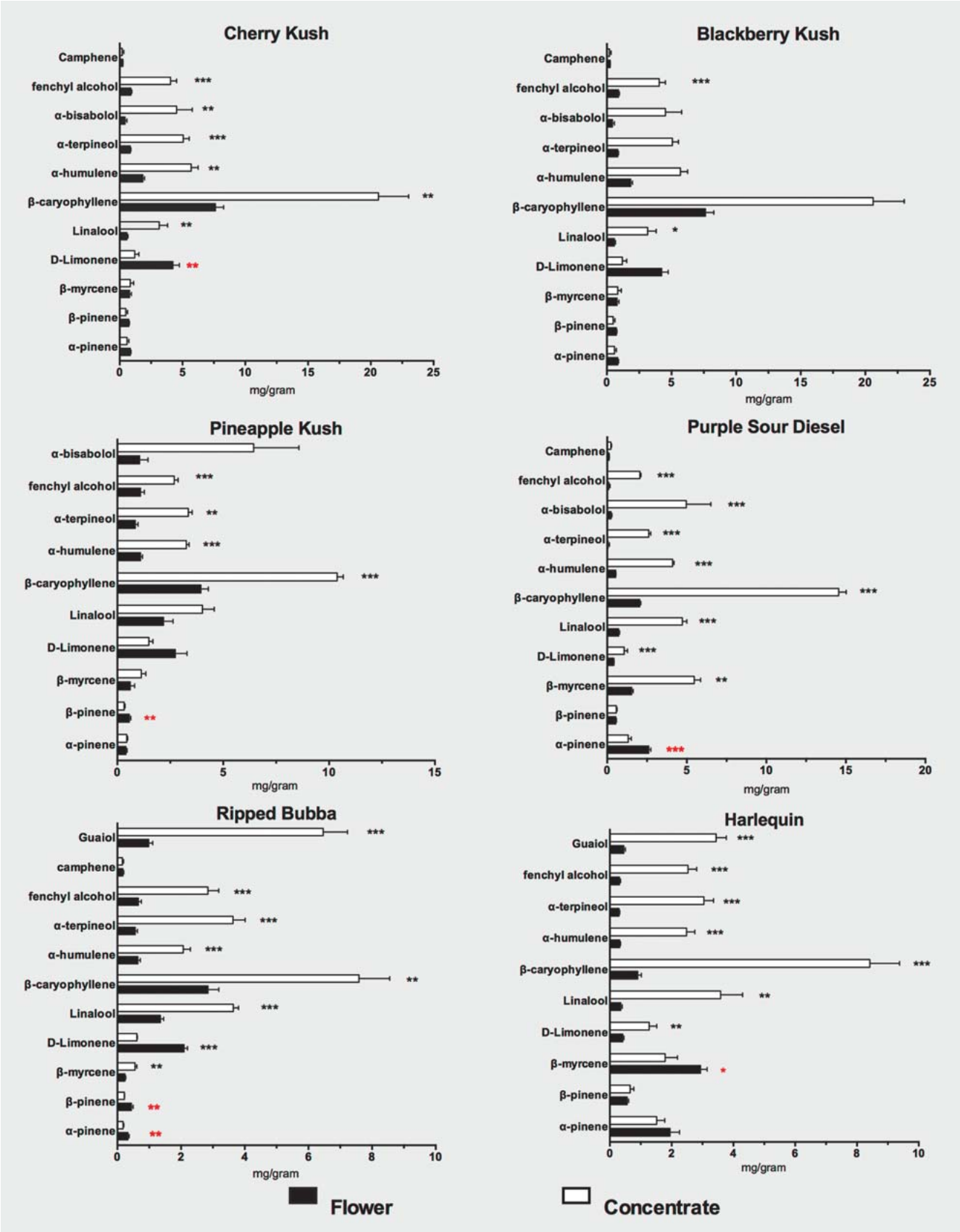
▶ **Fig. 1** (**A**) THC potency increased overall by a factor of 3.2 ($p < 0.05$) when comparing the flower chemotype to its respective SC-CO_2 extract (made from flower trim). Columns are the average of all chemotypes analyzed in triplicate and error bar is the standard deviation. (**B**) CBD potency increased by an overall factor of four when comparing flower chemotype to concentrate (no error bars as there was only one varietal with quantifiable CBD).

Discussion

The SC-CO_2 method to concentrate cannabis in this study demonstrates that this commercial extraction process did not replicate the flavor and fragrance of the starting material (flower sold by the same name.) Additionally, significant changes in potency occurred: THC content in a type 1 extract was significantly increased and this has important implications for inhalation of these products as it is largely unknown how either acute or chronic use at these doses affect either the endocannabinoid system or human health in general.

Loss of the monoterpene fraction, as shown in our analysis, constitutes a significant change between the chemotypes of cannabis flower and the concentrate. α-pinene has a relatively low vapor pressure of 4.75 mmHg at 25 °C, so at temperatures used in our SC-CO_2 process (up to 49 °C), there is the potential either for loss or for transformation products to appear [32]. Further, it has been reported that β-myrcene (a common abundant monoterpene in cannabis varietals) appears to undergo photolytic transformation in the processing of "hash." resulting in a new compound that has been named "hashishene" [33]. We did not analyze for any transformation products here and this is an area of research that is needed. While there is some data on the toxicology of essential oils and terpenoids, most of this research comes from studies of topical application, with less known about the toxicity of ingestion or inhaling these concentrated vapors. There is an ongoing need to evaluate upper limits for safety of inhalation for these concentrated natural products [34].

There is currently a void with regard to adequate definition of the products being sold as "medical marijuana" in the United States. The creation of these products has unfolded in a relative vacuum and at an unprecedented pace while federal restrictions have impeded the analytical community from creating standardized processes and protocols. Both standardized extraction



▶ **Fig. 2** Each flower with its predominant terpenoid chemotype is detailed. The terpenoid potency in the flower (black bar) is compared to the concentrate (white bar). Asterisks indicate significant differences, and red asterisks indicate flower having a higher concentration than the $SCCO_2$ concentrate. ($^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$)

▶ **Table 2** Monoterpene content of six cannabis chemovars.

Chemovar	α-pinene		β-pinene		β-myrcene		D-limonene	
	F	C	F	C	F	C	F	C
Cherry Kush	0.73 (± 0.09)	0.48 (± 0.11)	0.73 (± 0.14)	0.48 (± 0.08)	0.62 (± 0.54)	0.13 (± 0.03)	4.23* (± 0.50)	1.19 (± 0.33)
Blackberry Kush	1.900 (± 0.002)	0.31 (± 0.05)	0.25 (± 0.14)	0.39 (± 0.08)	0.60 (± 0.54)	0.14 (± 0.02)	1.23 (± 1.20)	1.09 (± 0.20)
Pineapple Kush	0.4 (± 0.3)	0.44 (± 0.60)	0.56* (± 0.04)	0.32 (± 0.05)	0.6 (± 0.2)	1.30 (± 0.21)	2.70 (± 0.55)	1.40 (± 0.19)
Purple Sour Diesel	2.60* (± 0.13)	1.30 (± 0.14)	0.54 (± 0.03)	0.57 (± 0.01)	1.53 (± 0.09)	5.46* (± 0.40)	0.41 (± 0.02)	1.06* (± 0.21)
Ripped Bubba	0.33* (± 0.02)	0.17 (± 0.01)	0.43* (± 0.05)	0.210 (± 0.003)	0.24 (± 0.02	0.55* (± 0.05)	2.09* (± 0.10)	0.60 (± 0.01)
Harlequin	1.94 (± 0.30)	1.51 (± 0.26)	0.56 (± 0.30)	0.65 (± 0.26)	2.90* (± 0.21)	1.78 (± 0.40)	0.40 (± 0.03)	1.27* (± 0.24)

Data are expressed as the mean in mg/g of starting material with standard deviation in parenthesis. Each sample was run in triplicate. Statistical significance is indicated by an asterisk ($p < 0.05$). F: flower; C: concentrate.

▶ **Table 3** Terpene alcohols and sesquiterpenes in six cannabis chemovars.

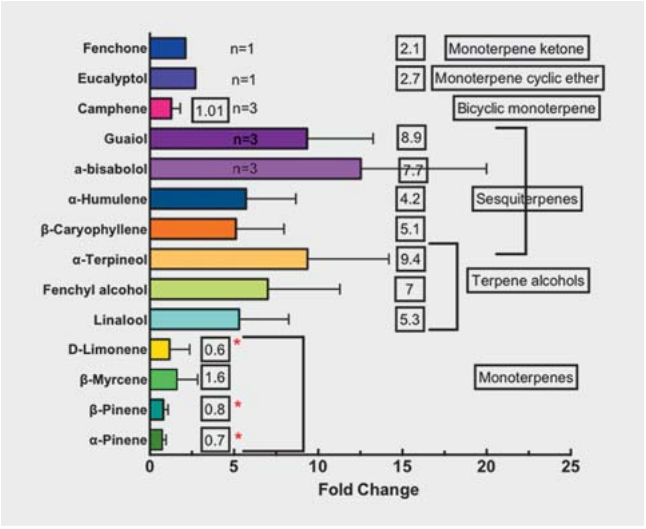
Chemovar	Linalool		Fenchyl alcohol		α-terpineol		β-caryophyllene		α-humulene		α-bisabolol	
	F	C	F	C	F	C	F	C	F	C	F	C
Cherry Kush	0.57 (± 0.08)	3.14* (± 0.66)	0.89 (± 0.08)	4.04* (± 0.47)	0.83 (± 3.50)	5.06* (± 0.46)	7.62 (± 0.64)	20.60* (± 2.40)	1.82 (± 0.13)	5.69* (± 0.55)	0.42 (± 0.15)	4.53* (± 1.25)
Blackberry Kush	0.18 (± 0.05)	0.93* (± 0.17)	0.35 (± 0.30)	4.01* (± 0.45)	0.25 (± 0.26)	4.65* (± 0.36)	0.33 (± 0.39)	2.10 (± 0.65)	0.17 (± 0.15)	1.65 * (± 1.43)	ND	ND
Pineapple Kush	2.10 (± 0.44)	4.00 (± 0.56)	1.09 (± 0.18)	2.68* (± 0.17)	0.84 (± 0.13)	3.35* (± 0.18)	3.94 (± 0.35)	10.38* (± 0.24)	1.09 (± 0.09)	3.23* (± 0.12)	1.04 (± 0.39)	4.96 (± 1.54)
Purple Sour Diesel	0.72 (± 0.05)	4.72* (± 0.28)	0.09 (± 0.09)	2.05* (± 0.33)	0.05 (± 0.08)	2.60* (± 0.38)	2.04 (± 0.06)	14.55* (± 0.46)	0.52 (± 0.01)	4.10* (± 0.09)	0.23 (± 0.05)	2.05* (± 0.90)
Ripped Bubba	1.35 (± 0.10)	3.64* (± 0.16)	0.65 (± 0.16)	2.84* (± 0.16)	0.55 (± 0.07)	3.63* (± 5.3)	2.80 (± 0.34)	7.59* (± 0.96)	0.64 (± 0.07)	2.06* (± 0.22)	ND	ND
Harlequin	0.35 (± 0.04)	3.58* (± 0.71)	0.31 (± 0.03)	2.53* (± 0.28)	0.29 (± 0.02)	3.94* (± 0.31)	0.90 (± 0.11)	8.40* (± 0.96)	0.32 (± 0.02)	2.48* (± 0.27)	ND	ND

Data are expressed as the mean in mg/g of starting material with standard deviations in parenthesis. Each sample was run in triplicate. Statistical significance is indicated by an asterisk ($p < 0.05$). F: flower; C: concentrate.

procedures and validated methodologies for quantification of compounds of interest have been lacking. Regardless, cannabinoid potency is routinely reported on certificates of analysis and product labels to 2–3 decimal points, lending an inference of precision that is may not be actual.

The market share for extracts in Washington State increased by 145.8% between 2014 and 2016, accounting for one-fifth of all sales [35]. These types of products have been reported elsewhere as having an 86% contamination rate with multiple chemical agents: insecticides, miticides, fungicides, and growth regulators [36]. The historic/legal framework has led to substantial knowledge gaps resulting in policies that fail to protect public health. For instance, initially Washington State regulations failed to require quality control testing for contaminants, such as pesticides, which can be concentrated in extracts [37].

Because this SC-CO_2 extraction protocol enhanced the potency of both cannabinoids and terpenoids in a differential fashion, further studies are necessary to determine the health-related consequences of inhaling concentrated cannabis extracts [38, 39]. A qualitative needs assessment of cannabis farm workers and owners found that many employers and employees identified terpenoids as the causative agent of their occupational allergies. Allergies presented in the form of respiratory distress and contact dermatitis. Currently, research does not identify the causative agent of occupational allergies in cannabis grow operations, attributing symptoms to generalized cannabis allergy [40].



▶ **Fig. 3** This graph depicts potency changes of 14 terpenoids across the six cannabis chemotypes. Bars are the average fold change between flower and concentrate across all samples and error bars are the standard deviation (n is labeled whenever < 6 samples contained this terpenoid).

Future research needs to be guided by optimized and standardized sample preparation and extraction processes, as well as by validated analytical methodologies by the commercial laboratories that analyze these products [24,41]. Once standard methods are widely adopted, human clinical studies can test for toxicity or for additive effects that terpenoids may have on cannabinoid pharmacology and pharmacodynamics (of CBD and THC) [42]. There may be medical benefit of terpenoids in oral products; however, due to significant first-pass metabolism, the effects may be more difficult to tease out than with inhalation [42–44]. Products sold in regulated U. S. retail markets and emerging markets worldwide need to be routinely tested and labeled with rank concentrations of terpenoids so that it is possible to discriminate differences in products beyond the common names and species labels of "sativa" and "indica" [25].

Limitations of this study include results that are only representative of a small Washington State analysis and that these results may not represent processes that others are using or any other products on the market. While the GC-MS terpenoid method may not be robust enough for some standards, we optimized the method internally and confirmed that the method produces repeatable results thus allowing for this intra-lab comparison/analysis.

Materials and Methods

Cannabis materials

Six chemovars of *C. sativa* flower were soil-grown in a 19-wk cycle, harvested after 8 wk of flowering, and cured to 12 % moisture content (PMB 53 Moisture Analyzer). The common names of the flower were Cherry Kush, Pineapple Kush, Purple Sour Diesel, Ripped Bubba, and Harlequin (laboratory accession IDs were STR 1378, 1382, 1382, 1383, 1394). The flower trim was collected for extraction when the dried flower was manicured, thus trim and flower were treated identically prior to extraction. Flower material and trim were collected and identified in May 2014 by Shawn DeNae (Washington Bud Company), the licensed grower in Washington State. As a result of legal restrictions applied to cannabis by the Controlled Substances Act (1970) to implement the Single Convention on Narcotic Drugs (1961), we were unable obtain voucher specimens from a major regional herbarium. We have insured the authenticity of the specimens by obtaining them directly from the production source.

SC-CO_2 extraction: 100 g of trim was prepared by grinding to a fine consistency (SharkNinja NJ600) thus increasing the surface area for SC-CO_2 to contact and vaporize all of the compounds. The powder was the packed into a 5-L extraction vessel (Eden Labs 2000 PSI SFE Hi-Flo). Cannabis was extracted in a closed-loop system that continued for about 6 h. For this study, temperature for the extraction vessel was 43 °C, separator temperature was 60 °C, and condenser was 4 °C. The system was pressurized to 1850 psi with internal temperature of 43 °C, well above the supercritical state of CO_2. Compounds were vaporized in the extraction vessel and pumped to the separator where the concentrated CO_2 was boiled off, leaving behind the cannabis extract. The gaseous CO_2 was then moved to the chiller coil and condensed back into a liquid in the accumulator for re-use. To remove residual water, the product was purged in a vacuum oven for 24 h at − 28 mmHg and 49 °C. As water was removed, oil and wax recombine creating a viscous sticky product, amber in color.

Sample preparation

Ground flower material (0.25 g) or SC-CO_2 extract (0.15 g) (n = 3) was dissolved in 10 mL of 1 : 1 of HPLC-grade dichloromethane/HPLC-grade methanol (DCM/M) (Sigma-Aldrich). The mixture was vortexed for 1 min and then degassed by briefly opening the tube and recapping. The mixture was sonicated for 30 min (degassed again at 15 min time point) and then centrifuged at 9000 ×g for 3 min. The supernatant was collected and diluted in DCM/M 1 : 5 for flower, 1 : 10 for concentrate, in a micro centrifuge tube, and centrifuged at 9000 ×g for 3 min. Sample was filtered using a 45-µm PTFE syringe filter.

GC/MS

Terpenoids were separated on an Agilent 5890 GC with a HP5972 Mass Analyzer, using an HP autosampler. The mass spectra were generally recorded over 40–500 amu that revealed the total ion current chromatograms. Column: Restek Rtx-624 column (30 m × 0.25 mm × 1.40 µm). Injection: Samples were directly injected (1 µL, fast injection) into the inlet at a flow rate of 1.6 mL/min. The temperatures of the injector, transfer line, and ion source were maintained at 210 °C, 210 °C, and 200 °C, respectively. Carrier gas was 99.999 % grade He. The gradient was begun with an initial oven temp of 40 °C at 0.75 min hold and a ramp at 6 °C per minute up to 230 °C hold for 4 min. Peak identities were assigned unambiguously by comparison to those of the authentic standards by retention time and mass spectra. Spiked "unknowns" were analyzed in triplicate for recovery at 2, 10, and

15 µg/mL prior to sample quantification (▶ **Table 1**). Response of the mass spectra was monitored by the use of a control standard using 10 µg/mL of the standards. There was a solvent delay of 12.5 min. Here we report only the 15 most abundant compounds identified by MS occurring well above the limit of detection in mg/gram of starting material. The method was developed and determined to be "fit for purpose" and sufficient for comparison in a single laboratory between two matrices.

Terpenoid reference standards

Flower and concentrate were analyzed for 42 terpenoids (100 µg/mL in methanol) from a common stock solution (SPEX CANTERP-MIX 1 and 2) and quantified using standard curves prepared gravimetrically in DCM/M. To measure recovery, the reference standards were spiked at low, medium, and high levels onto dried, ground straw (0.25 g) and extracted in DCM/M. Calibrations standards were stored at − 20 °C in the dark and tested at regular intervals to assess stability in solution. The diluent solutions for a six-point standard curve were determined at concentrations of 2, 3, 4, 5, 10, 15, and 30 µg/mL, run at the same time as the unknowns, in triplicate and repeated on three separate days. This method was optimized using AOAC international guidelines. Certificates of analysis were used for compound identification and peak identities were assigned unambiguously by comparing retention time and mass spectra to the standards. We report terpenoids that were expressed in the plant above 200 µg/g and required correlation coefficients ≥ 0.99 for quantification. The calibration curves were plotted and the slope and y-intercept for each terpenoid were used for linear regression analysis. Additional data on the method are provided as Supporting Information in **Table 1S**.

HPLC/DAD

Cannabinoids were quantified using a previously validated method by DeBacker et al., with minor modifications [45]. Briefly, 0.25 g of plant material or 0.15 g of concentrate was added to 10 mL isopropanol and sonicated for 30 min at 30 °C. The sample was diluted 1 : 40 with mobile phase (acetonitrile/water) and filtered. Thirty microliters were injected onto an Agilent SDB-C18 Rapid Resolution Column (4.6 × 50 mm × 3.5 µm) at a flow rate of 0.3 mL/min, running isocratic. Full spectra were recorded from 200–400 nm. Methyl paraben was used as an internal standard. Neutral cannabinoids were quantified at 200 nm and acid cannabinoids at 270 nm: CBDA, CBD, THCA, THC, CBN, CBG (Lipomed AG). The individual cannabinoid content is calculated according to the following equation:

$$W_{CBX(T)} = \frac{C_{CBX(T)} \times 10^6 \times V_{sample} \times D}{m_{sample}} \times 100\% \quad (1)$$

Total content in the sample (C_{max}) is calculated as a sum of the concentrations of the neutral cannabinoids (C_{CBX}) and the acidic (C_{CBXA}) forms. A 0.877 conversion factor allows for adjustment of acidic components after decarboxylation.

Statistical analysis

T-test analysis was performed using Graph Pad Prism. Statistical significance determined using the Holm-Sidak method, with alpha = 5.000 %. Each row was analyzed individually, without assuming a consistent standard deviation (SD).

Supporting information

Additional data on the method are provided as Supporting Information.

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Conflict of Interest

There are no conflicts of interest to divulge.

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