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Influence of Ethanol Concentration on the Extraction of Cannabinoid and Volatile Compounds for Dry-Hemped Beer

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Abstract: Beer is one of the most widely consumed beverages in the world. Since the legalization of low-delta-9-tetrahydrocannabinol (THC) (<0.03%) *Cannabis sativa* in the United States, this controversial plant is being looked upon as a potential flavor additive for use in beer. *Cannabis sativa* shares similar aromatic and flavor characteristics to that of hops (*Humulus lupulus*). This study was designed to determine the influence ethanol concentration has on the cannabinoid and volatile compounds found in beer. Three experimental beers with varying ethanol (3, 6, and 9% ABV) concentrations were used for this experiment. Using dry-hopping practices and dosing from commercial brewing techniques, hemp was placed in the beer for five days at 25 °C. Cannabinoids and volatile compounds were analyzed following the completion of the dry-hemping process. Statistical differences were observed only for the cannabinoid, tetrahydrocannabinolic acid (THCA), while, no other individual cannabinoid or the summation of the total cannabinoids were statistically different. Volatile compounds were analyzed using gas chromatography–mass spectrometry (GC-MS). Statistical differences were observed between the monoterpenes and the sesquiterpenes volatile compound groups. In summary, ethanol concentration appears to have no impact on the extraction efficiency of cannabinoids but does appear to influence volatile compounds when hemp is added to beer through the dry-hemping process.

Keywords: hemp; brewing; volatile compounds; cannabinoids



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1. Introduction

In recent years, industrial hemp (*Cannabis sativa*) has garnered increased attention following the enactment of the Agricultural Improvement Acts of 2014 and 2018, passed by the United States Congress [1]. The European Union passed a similar article allowing farmers to grow industrial hemp under Articles 32(6), 35(3), and 52 of Regulations (EU) 1307/2013 [2]. Following this act, *Cannabis* in the United States was categorized into two groups based on the concentration of delta-9-tetrahydrocannabinol (Δ^9 -THC), industrial hemp (<0.3%; federally legal), and drug-type marijuana (>0.3%; federally illegal) [1]. This reclassification has ignited a surge of interest in the potential applications of industrial hemp, particularly in states like Florida, where it is likely to become a valuable alternative crop.

There are over 30 European, Asian, North and South American countries legally producing industrial hemp. Canada, China, and the European Union are the top three global producers [3]. Canada is the world's top producer of hemp-based foods such as

hemp protein powder, hemp oil, and seeds, while China is the leading producer of hemp fiber accounting for over 50% of the world's production [3,4]. However, any form of hemp production is still highly regulated or illegal in much of the world, specifically in Asia [5].

Cannabis sativa has been cultivated throughout history for grain (seed), fiber (stems), and for its secondary metabolites (essential oils) [1]. In many countries, the use of *C. sativa* for industrial commercial applications was limited throughout most of the 20th century due to strict regulations banning its cultivation, sale, and use. Modifications to existing regulations, recently enacted in the U.S. and several European countries (Germany [1], the United Kingdom [6], and France [7]), have eased restrictions on *C. sativa* and have resulted in increased interest in the commercial application of *C. sativa* products. Given its regulated history, the presence of Δ^9 -THC in *C. sativa* has made even industrial hemp a somewhat controversial plant. However, coupled with hops (*Humulus lupulus*), they represent an important group of plants within the pharmaceutical, agricultural, and industrial industries [8,9]. Hops are the closest relatives of *C. sativa* and share many of the same characteristics. Hops and hemp both belong to the plant family Cannabaceae, in the order of Rosales [10]. Like hops, *C. sativa* possesses a well-known and unique aromatic profile that is generally well-liked and easily discernible [11]. Researchers Gibert and DiVerdi (2018) conducted a sensory study to determine whether untrained panelists could differentiate between several different hemp cultivars. The study concluded that untrained panelists can easily tell the difference between them [11].

In 2018, New Belgium developed a beer called "The Hemperor IPA". Hemperor is a traditional IPA (Indian pale ale) with IBUs (international bitterness units) around 55. The beer was brewed using Cascade, Nugget, Simcoe, and HBC 522 hops, along with hemp hearts and other natural flavors [12]. Due to legality reasons, New Belgium was unable to utilize any other part of the hemp plant [8]. Tasting notes, which can be found on their website, describe the beer as having a pungent aromatic profile coupled with herbal, pine, and low citrus notes [12]. The limited use of hemp in beer has been focused primarily on complementing or adding to flavors introduced by hops. There have been no published investigations into producing beers with hemp as the primary aroma. Hemp and hops share the same botanical lineage and have been shown to share similar aromatic properties. This could be important, especially for industrial hemp growers, who are looking for additional revenue streams for their product outside of medicinal uses.

Thus, building on the similarities between hemp and hops, there is the potential for hemp to enhance the flavor and aroma profiles of fermented beverages such as beer. The objective of this work was to determine the impact of ethanol concentration on the extraction of cannabinoids and flavor compounds found in hemp.

2. Materials and Methods

2.1. Plant Material

Ninety *C. sativa*, hereafter referred to as hemp, plants were vegetatively propagated on 25 May 2023 from genetically identical 'Wife' mother stock plants maintained at the University of Florida's Mid-Florida Research and Education Center in Apopka, FL. After excess leaves were removed from plant propagules, stems were dipped into rooting hormone gel (Clonex Rooting Gel, Growth Technology Ltd., Somerset, UK) containing 3.1 g/L indole-3-butyric acid (IBA), then inserted into sphagnum peat moss cubes (Root Riot Plant Starter Cubes, Hydrodynamics International Inc., Lansing, MI, USA). Cubes were placed in 40-cell propagation trays (Gardzen Plant Trays, Gardzen LLC, Rowland Heights, CA, USA) and covered with plastic humidity domes. Cuttings were hand-watered as needed and grown at 25 °C under a 24 h photoperiod until roots were visible at the bottom of the cells (~12 d). Rooted cuttings were transplanted into 10.2 cm containers (STD0400, ITML Horticultural Products Inc., Brantford, Ontario, CA, USA) containing high-porosity peat-based media (Pro-Mix HP Mycorrhizae, Premier Tech Horticulture, Quakertown, PA, USA) and moved into a greenhouse with supplemental lighting under an 18 h light and 6 h dark photoperiod to maintain vegetative growth. The plants were hand-watered as needed and

were fertigated twice with 20-20-20 nitrogen, phosphorous, and potassium (NPK) fertilizer (Peters Professional, ICL Specialty Fertilizers, Dorchester County, SC, USA) at a rate of 150 ppm nitrogen before being transferred to the field on 21 June 2023. Plants were irrigated via drip lines for one hour each morning. Controlled release fertilizer (150-0-100 NPK; Polyon, Harrell's LLC, Lakeland, FL, USA) was applied to the base of the plants to ensure plant nutritional needs were sufficient to support growth and development. On 22 September 2023, apical floral samples (cola) measuring approximately 15 cm were harvested from the plants and dried at 60 °C in a food dehydrator for three days. Following drying, hemp floral samples were placed in a sealed plastic sample bag, transferred to the University of Florida in Gainesville, FL, and stored in a secure, cool environment.

2.2. Yeast Propagation

Saccharomyces pastorianus (Lallemand—Montréal, QC, Canada) was propagated prior to the start of this experiment using 1 L of 10 °Plato dry malt extract (Briess DME, Chilton, WI, USA) in a 1 L Erlenmeyer Flasks incubated on a shaker table at 24 ± 3 °C at 100 rpm. The yeasts were allowed to propagate for 5 days before use [13].

2.3. Yeast Cell Count and Viability

Yeasts cells count and viability were measured using a Bio-Rad TC20 Automated Cell Counter (Bio-Rad Laboratories, Hercules, CA, USA).

2.4. Yeast and Fermentation

Saccharomyces pastorianus was pitched at a rate of 1.0 million cells per mL °Plato [13].

2.5. Experimental Beer

A 11.4 L wort was brewed with a specific gravity of 1.047 (11.6 °Plato) and was used throughout the entire project. The wort was prepared using 1.5 kg of Pilsner Liquid Malt Extract (Briess Malting and Ingredient Co, Chilton, WI, USA). The wort was hopped to twenty IBUs using magnum hops to provide antiseptic properties to the beer. The wort was boiled for thirty minutes for sterilization purposes and then cooled to fermentation temperature (21 °C) and manually aerated prior to pitching. The wort was then inoculated with 1.0 million cells °Plato using *Saccharomyces pastorianus*. A single fermentation vessel was used to ensure a consistent volatile profile of the base beer over several experiments. The beer was allowed to ferment at 21 °C for two weeks. Following the completion of the fermentation process, the beer was cold crashed in a 4 °C refrigerator for five days. After cold crashing, the experimental beer was split in half for multiple studies.

2.6. Gravity Measurements

Original and final gravity measurements for the wort were carried out by utilizing the ASBC (American Society of Brewing Chemists) official method Beer-4-E (instrumental method for Alcohol and Original Gravity) using the Anton Paar (Gesellschaft mit beschränkter Haftung) DMA 35 Standard portable density meter.

2.7. Preparation of Different ABV (Alcohol by Volume) Beers

The original experimental beer had an alcohol concentration of 5% ABV. The 5.7 L of finished experimental beer was split into three different beer solutions and supplemented with ethanol to produce different alcohol content by volume. Table 1 shows the dilution scheme used to prepare the different concentrations. The solutions were prepared aiming to maintain a constant dilution factor of the beer to ensure that the volatile profile was affected equally across all sample types due to the dilution to meet our desired alcohol content.

Table 1. Dilution scheme to achieve desired alcohol content.

Alcohol Content by Volume (ABV)	Beer (mL)	Ethanol (70%) (mL)	Distilled Water (mL)	Final Volume (mL)
3%	900	0	600	1500
6%	945	85	470	1500
9%	950	125	425	1500

2.8. Dry-Hemping

Following the fermentation process and ABV (alcohol content by volume) adjustment, nine separate 500 mL dry-hemping vessels were utilized, allowing for each ABV to be run in triplicate. Each beer was dry-hemped with 0.53 g of Wife hemp flower material. Hemp was placed into 7 × 9 cm disposable tea bags (Doloburn, Hong Kong, China) and allowed to seep for five days. After five days, the hemp material was removed, and beer samples were analyzed or stored at 5 °C until further analysis was completed.

2.9. pH

pH was measured using an Orion pH meter (Waltham, MA, USA). Before use, the pH meter was calibrated using a three-point calibration curve.

2.10. Identification and Quantification of Cannabinoids

Hemp beer samples were analyzed for cannabichromene (CBC), cannabichromenic acid (CBCA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabicyclol (CBL), cannabinol (CBN), delta 8-tetrahydrocannabinol (Δ^8 -THC), Δ^9 -THC, tetrahydrocannabinolic acid (THCA), and tetrahydrocannabivarin (THCV) using an in-house ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method [14]. In brief, hemp-beer samples (100 μ L) were diluted with a diluent solution (95:5 methanol/water with 0.005% formic acid), spiked with internal standards (IS, Δ^9 -THC-*d*3, and CBD-*d*3; each 500 ng/mL), vortex mixed, filtered through a multiscreen Solvinert (Millipore, St. Louis, MO) 96-well filter plate (0.45 μ m) and injected onto Waters Acquity UPLC I Class coupled with Xevo® TQ-S micro triple quadrupole mass spectrometer. An Acquity BEH C18 column (2.1 × 100 mm, 1.7 μ m, Waters Corp, Milford, MA) was used for analytical separation in gradient mode. The optimized linear gradient program (0.35 mL/min) with mobile phases (A) 0.1% formic acid in water and (B) methanol/acetonitrile (50:50 *v/v*) was as follows: 0–30 s, 18% A and 82% B; 30 s–5.5 min, 100% B (linearly increased), and 5.5–6.0 min, 82% B. Calibration (CS) and quality control (QC) standards were prepared using blank beer (Budlight) spiked with known amounts of standard stock solutions containing all thirteen cannabinoids and IS. Blank beer samples with or without IS were also included along with CS, QC, and test samples. Multiple reaction monitoring with electrospray ionization in positive mode was utilized for the detection of cannabinoids and IS in beer samples. Data acquisition was conducted using MassLynx® 4.2 software, while TargetLynx™ facilitated the quantification of each cannabinoid (Waters, Milford, MA, USA).

2.11. Essential Oil Extraction of the Volatile Compounds

The essential oils and volatile organic compounds (VOCs) were quantified using ASBC's official method, Hops-13, with minor adjustments. The hemp flower material (Wife strain) was separated from the stems and ground in a consumer-grade coffee grinder for 20 s, ensuring a consistent sample. Five grams of this ground hemp were placed into a 1000 mL evaporation flask (Büchi, Flawil, Switzerland), followed by the addition of 150 g of deionized (DI) water. A Büchi Rotavapor® (R-300) vacuum distillation unit was employed to characterize the VOCs in the hemp samples. The vacuum pressure was set at 25 inHg, resulting in a boiling point of approximately 55 °C for the ground hemp and water mixture. The evaporation flask rotated at approximately 200 RPM to optimize heat transfer and

ensure uniform mixing. The water bath temperature was initially set at 69 °C for about an hour and then raised to 90 °C for an additional 30 min or until at least 75% of the water mass was recovered. Fifty milliliters of the distilled solution were transferred into a 100 mL separatory funnel for further processing.

A Folch extraction method was used to isolate the lipid fraction. A 2:1 chloroform to methanol mixture (5 mL) was added to the separatory funnel containing the hemp distillate. Additionally, 0.5 mL of a 5% NaCl solution was introduced to remove any remaining water from the lipid portion. Following fractionation, 2.5 mL of the lipid section was transferred to a 10 mL clear headspace vial with a screw cap (Thermo Fisher Scientific, Waltham, MA, USA). For internal standardization, 5 µL of 2-octanol at a concentration of 328 mg/L was added to the 2.5 mL lipid sample. Within 24 h, the hemp oil/lipid sample was analyzed using GC-MS.

2.12. Extraction of the Volatile and Semi-Volatile Compounds

The extraction of the volatile and semi-volatile compounds found within the beer was carried out following the method described by Nemenyi et al., 2024 [15].

2.13. Gas Chromatography—Mass Spectrometry (GC-MS)

The GC-MS method utilized for the separation of the volatile and semi-volatile compounds followed the method previously described by Nemenyi et al., 2024 [15].

2.14. Volatile Analysis of the Essential Oil Using GC-MS

A 10 µL sample was injected into Shimadzu gas chromatograph (GC) 2010 Plus Series mass spectrometer detector (MSD) QP2010 SE (Columbus, Maryland). The injection port was set to 250 °C, and all injections were made in split mode using a 15:1 ratio with a narrow bore and deactivated glass insert. A 4.5 min solution delay was utilized to protect the detector. Volatile compounds were separated using a nonpolar Zebron—5% phenyl-arylene/95% dimethylpolysiloxane ZB-5MS (ZB; 30 m × 0.25 mm id × 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 2.0 mL/min (linear velocity 53.8 cm/s). The GC program utilized for separation and analysis followed the same program as previously described above [16].

2.15. Statistics

Statistical analyses were conducted using coding for R (R Project for Statistical Programming, Vienna, Austria). Statistical calculations were carried out using a one-way analysis of variance (ANOVA) and paired with Tukey's honestly significant difference test.

A matrix dataset constructed from the concentrations of the different volatile compound profiles from the different beers with different ethanol concentrations was used for principal component analysis (PCA). Python (3.7.3) (Wilmington, DE) was implemented with scikit-learn (1.0.1), pandas (1.3.4), NumPy (1.21.4), and plotly (5.3.1) libraries for three-dimensional PCA and visualization.

3. Results and Discussion

This section discusses the impact of increasing ethanol concentration on the incorporation of cannabinoids and composition of volatile compounds in dry-hemped beers.

3.1. Cannabinoids

Alpha acids and cannabinoids are both hydrophobic compounds due to their lipid nature [9]. These characteristics make incorporating these compounds difficult into a beverage primarily composed of water like beer [9]. There are over five hundred phytochemicals found in cannabis plants, with at least 120 of them being cannabinoids [9]. The cannabinoids studied are as follows: CBC—cannabichromene, CBCA—cannabichromenic acid, CBD—cannabidiol, CBDA—cannabidiolic acid, CBDV—cannabidivarin, CBG—cannabigerol, CBGA—cannabigerolic acid, CBL—cannabicyclol, CBN—cannabinol, 8THC— Δ^8 -tetrahydrocannabinol,

9THC— Δ^9 -tetrahydrocannabinol, THCA—tetrahydrocannabinolic acid, and THCV—tetrahydrocannabivarin [17]. Table 2 shows the concentrations (ng/mL) of individual cannabinoids found in beer with different ethanol concentrations.

Table 2. Individual cannabinoid concentration based on ethanol concentration (ABV) in dry-hemped beer samples.

Cannabinoid	Concentration (ng/mL)		
	3% ABV	6% ABV	9% ABV
CBC	--	--	--
CBCA	12.75 $\pm$ 0.6	13.4 [†]	10.1 [†]
CBD	--	--	--
CBDA	44.5 $\pm$ 5.7 ^A	15.3 ^{B, †}	11.45 $\pm$ 1.2 ^B
CBDV	--	--	--
CBG	--	--	--
CBGA	--	--	--
CBL	--	--	--
CBN	--	--	--
8THC	--	--	--
9THC	--	--	--
THCA *	21.4 $\pm$ 10.1	22.4 $\pm$ 5.5	20.6 $\pm$ 0.95
THCV	--	--	--
Total *	74.5 $\pm$ 21.3	31.93 $\pm$ 21.93	31.6 $\pm$ 10.51

N = 3; means $\pm$ standard deviation (STD) indicates below lower limit of quantification (BLLOQ, 10 ng/mL).

* indicates no statistical differences were observed ($p > 0.05$). Values bearing different letters are statistically significant ($p < 0.05$). [†]: only one sample contained any of the cannabinoid, so no STD reported CBC—cannabichromene, CBCA—cannabichromenic acid, CBD—cannabidiol, CBDA—cannabidiolic acid, CBDV—cannabidivarin, CBG—cannabigerol, CBGA—cannabigerolic acid, CBL—cannabicyclol, CBN—cannabinol, 8THC— Δ^8 -tetrahydrocannabinol, 9THC— Δ^9 -tetrahydrocannabinol, THCA—tetrahydrocannabinolic acid, and THCV—tetrahydrocannabivarin.

A statistically significant difference ($p < 0.05$) was only seen for THVC; however, due to only a single sample containing CBCA, further analysis with additional samples is required to determine if true statistical differences do exist. However, no statistical differences were observed between CBCA, THCA, and the overall total cannabinoid concentration. Koenigs reported that the extraction efficiency of cannabinoids increases with ethanol [8]. However, based on these results, the opposite was found for cannabinoids. Whole hemp flowers were used for this experiment, which could have impacted the cannabinoids' availability for extraction, thereby decreasing the efficiency. It would be interesting to test if the use of ground hemp material would show similar results, since coarsely ground material would have an increased surface area, potentially allowing the cannabinoids more opportunity to be available for extraction [8].

3.2. Volatile Composition

Beers with varying alcohol percentages were also investigated for changes in the composition of volatile compounds present post-dry-hemping. Table 3 shows the complete aromatic profile of the different beers based upon their different alcohol concentrations by volume. The base beer was also analyzed to determine which compounds were present in the beer before dry-hemping, as well as which compounds decreased because of the dilutions made to achieve the various alcohol concentrations. The volatile composition of the hemp plant material was also analyzed to determine if the compound identified was a product of fermentation or from the hemp plant. A one-way ANOVA coupled with Tukey's HSD was run to determine statistical differences between the dry-hemped beer. Statistical differences were observed between all the groups except alcohols and terpenes.

Table 3. Volatile composition of base beer and the different alcohol concentrations (3%, 6%, and 9%), and the hemp variety essential oil extracted. All determined compounds also include an odor descriptor when known.

Compound	LRI	Odor Descriptors *	Base Beer	Approximate Concentrations (mg/L)			Cannabis sativa L. ‘Wife’ EO
				3%	6%	9%	
Acids							
Octanoic acid	1182	Goaty	0.50 ± 0.09	0.12 ± 0.05	0.21 ± 0.11	0.59 ± 0.12	----
Nonanoic acid	1263	Green, fat	0.13 ± 0.11	----	0.06 ± 0.04	0.11 ± 0.08	----
Decanoic	1354	Caprylic	0.90 ± 0.30	----	0.12 ± 0.08	0.28 ± 0.12	----
Subtotal €			1.48 ± 0.32	0.12 ± 0.05 ^B	0.38 ± 0.2 ^{AB}	0.77 ± 0.19 ^A	0 ± 0
Alcohols							
3-Methylbutanol (Isoamyl alcohol)	773	Whisky	11.33 ± 2.78	7.93 ± 3.07	7.73 ± 3.06	8.52 ± 0.08	----
2-Methylbutanol (Active amyl alcohol)	784	Malt	----	----	----	----	2.36 ± 0.13
3-Hexenol	868	Grassy	----	----	----	----	13.15 ± 0.64
1-Hexanol	875	Green	----	0.10 ± 0.01	0.12 ± 0.02	0.09 ± 0.01	194.28 ± 5.90
Heptanol	976	Solvent	----	0.03 ± 0.01	0.06 ± 0.03	0.06 ± 0.01	3.77 ± 0.17
1-Octen-3-ol	986	Green	----	----	----	----	3.60 ± 0.12
2-Octen-4-ol	1000	Fruity	----	----	----	----	4.26 ± 0.27
Benzyl alcohol	1038	Almonds	----	----	----	----	9.62 ± 2.68
1-Octanol	1070	Orange, rose	0.25 ± 0.09	0.21 ± 0.03	0.27 ± 0.08	0.32 ± 0.02	----
Phenylethyl Alcohol	1111	Rose, honey-like	5.99 ± 1.37	1.56 ± 0.05	2.93 ± 1.36	4.77 ± 1.12	35.36 ± 6.14
1-Decanol	1265	Floral, fruity	0.20 ± 0.02	0.05 ± 0.04	0.04 ± 0.01	0.06 ± 0.01	----
Subtotal ^G			17.78 ± 4.24	9.89 ± 3.04 ^A	11.1 ± 4.59 ^A	13.79 ± 1.19 ^A	266.4 ± 9.66
Aldehydes							
Hexanal	819	Green, leafy	----	----	----	----	16.58 ± 0.65
2-Hexenal	864	Apple, green	----	----	----	----	34.13 ± 1.22
Heptanal	909	Unpleasant	----	----	----	----	22.8 ± 0.89
Benzeneacetaldehyde	1045	Berry, honey	0.03 ± 0.01	----	----	0.04 ± 0.01	6.01 ± 0.38
p-Tolualdehyde	1081	Floral	0.04 ± 0.01	----	----	----	----
Nonanal	1100	Cardboard	0.30 ± 0.20	0.33 ± 0.22	0.21 ± 0.03	0.22 ± 0.04	----
Subtotal ^G			0.36 ± 0.18	0.33 ± 0.22 ^A	0.21 ± 0.03 ^A	0.25 ± 0.05 ^A	70.71 ± 13.88
Esters							
Isoamyl acetate	883	Fruity	14.02 ± 3.99	6.34 ± 0.68	7.76 ± 0.96	8.06 ± 0.63	----
Ethyl hexanoate	1002	Apple	0.91 ± 0.61	0.32 ± 0.08	0.34 ± 0.04	0.60 ± 0.15	----
Hexyl acetate	1018	Sweet	----	0.14 ± 0.06	0.13 ± 0.06	0.09 ± 0.05	5.80 ± 0.16
Ethyl heptanoate	1093	Fruity	0.07 ± 0.06	----	----	0.06 ± 0.02	----
Hexyl isobutyrate	1145		----	0.07 ± 0.06	0.05 ± 0.01	0.05 ± 0.01	----
Ethyl benzoate	1171	Chamomile, flower,	0.09 ± 0.02	----	0.07 ± 0.03	0.21 ± 0.02	----

Table 3. Cont.

Compound	LRI	Odor Descriptors *	Base Beer	Approximate Concentrations (mg/L)			Cannabis sativa L. ‘Wife’ EO
				3%	6%	9%	
Hexyl butanoate	1191	Apple peel	----	0.34 ± 0.02	0.62 ± 0.26	0.72 ± 0.63	85.9 ± 8.68
Ethyl octanoate	1197	Apple, sweet	6.58 ± 2.56	1.93 ± 0.22	3.33 ± 0.33	4.56 ± 0.26	----
Octyl acetate	1209	Coconut	0.08 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	----
Phenethyl acetate	1250	Rose, honey	4.84 ± 1.52	0.78 ± 0.09	1.68 ± 0.82	2.80 ± 0.49	----
Ethyl nonanoate	1284	Fruit	0.10 ± 0.09	0.01 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	----
2-Methylpropyl octanoate	1333		0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	----
Ethyl 9-decenoate	1374		0.05 ± 0.02	0.02 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	----
Ethyl decanoate	1382	Caprylic, fruit	3.80 ± 2.06	0.95 ± 0.12	1.97 ± 0.17	2.96 ± 0.47	----
3-Methylbutyl octanoate (Isoamyl octanoate)	1433		0.04 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	----
Ethyl dodecanoate		Soapy	0.54 ± 0.11	0.13 ± 0.09	0.23 ± 0.04	0.38 ± 0.08	----
Subtotal F			30.97 ± 10.45	11.33 ± 0.63^B	17.67 ± 2.86^A	20.66 ± 0.97^A	85.9 ± 8.68
<i>Terpenes</i>							
Fenchol	1115	Camphor	----	0.59 ± 0.12	0.76 ± 0.24	0.90 ± 0.07	297.76 ± 3.10
2-Pinanol	1125		----	0.03 ± 0.01	0.05 ± 0.01	----	253.86 ± 5.40
Ipsdienol	1141		----	0.09 ± 0.01	0.16 ± 0.09	0.21 ± 0.02	610.71 ± 6.81
(-)-Borneol	1175	Camphor	----	0.28 ± 0.05	0.37 ± 0.14	0.45 ± 0.08	235.07 ± 15.34
(+)-Borneol	1206	Piney, camphor	----	0.07 ± 0.01	0.15 ± 0.08	0.19 ± 0.04	215.83 ± 4.44
Guiaol			----	0.26 ± 0.12	0.19 ± 0.05	0.2 ± 0.08	271.96 ± 13.61
Subtotal G			0 ± 0	1.30 ± 0.30^A	1.66 ± 0.49^A	1.98 ± 0.15^A	1885.19 ± 17.58
<i>Monoterpenes</i>							
α-Pinene	936	Pine	----	0.21 ± 0.03	0.27 ± 0.15	0.08 ± 0.02	11.59 ± 0.50
β-Pinene	980	Pine, resin	----	0.19 ± 0.05	0.25 ± 0.17	0.12 ± 0.08	12.55 ± 1.80
β-Myrcene	993	Herb	----	1.86 ± 0.29	1.39 ± 0.86	0.98 ± 0.22	62.57 ± 1.47
Limonene	1032	Lemon	----	0.27 ± 0.05	0.22 ± 0.03	0.15 ± 0.02	12.53 ± 0.44
Eucalyptol	1035	Camphor	----	0.27 ± 0.08	0.32 ± 0.07	0.39 ± 0.02	343.18 ± 6.37
L-Fenchone	1088		----	0.17 ± 0.04	0.13 ± 0.03	0.12 ± 0.02	22.77 ± 1.10
Linalool	1097	Citrus	0.29 ± 0.08	1.89 ± 0.19	2.68 ± 0.98	3.01 ± 0.2	1201.29 ± 31.09
Pinocarvone	1163	Flower	----	0.04 ± 0.04	0.02 ± 0.01	0.03 ± 0.01	11.73 ± 3.46
L-4-terpineneol	1182	Turpentine, nutmeg	----	0.11 ± 0.09	0.45 ± 0.26	0.99 ± 0.49	80.11 ± 19.18
α-Terpineol	1198	Pine	0.25 ± 0.02	----	0.42 ± 0.22	0.51 ± 0.12	612.98 ± 8.18
Carveol	1219	Caraway, solvent	----	----	0.03 ± 0.01	0.03 ± 0.01	42.74 ± 4.85
Citronellol	1225	Floral	0.13 ± 0.01	0.11 ± 0.03	0.14 ± 0.05	0.21 ± 0.04	67.24 ± 5.45
Citral	1246	Lemon	----	----	0.02 ± 0.01	0.02 ± 0.01	----
Methyl geranate	1308		0.04 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	----

Table 3. Cont.

Compound	LRI	Odor Descriptors *	Base Beer	Approximate Concentrations (mg/L)			<i>Cannabis sativa</i> L. ‘Wife’ EO
				3%	6%	9%	
Subtotal ¥			0.42 ± 0.03	5.36 ± 0.46 ^B	6.12 ± 0.78 ^{AB}	6.62 ± 0.42 ^A	2481.3 ± 35.83
<i>Sesquiterpene</i>							
Hotrienol	1155	Hyacinth	----	0.07 ± 0.01	0.14 ± 0.08	0.21 ± 0.03	----
Caryophyllene	1415	Woody	0.01 ± 0.01	0.29 ± 0.05	0.23 ± 0.06	0.23 ± 0.03	86.11 ± 3.41
α-Guaiene	1430		----	0.01 ± 0.01	0.01 ± 0.01	----	----
α-Humulene	1455	Wood	0.38 ± 0.25	0.20 ± 0.01	0.20 ± 0.05	0.21 ± 0.05	35.76 ± 2.10
α-Amorphene	1478		----	0.03 ± 0.02	0.03 ± 0.03	0.03 ± 0.01	----
β-Selinene	1492	Herb	----	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	----
β-Bisabolene	1508	Balsamic	----	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	12.03 ± 1.38
Humulene Epoxide I			0.13 ± 0.05	0.07 ± 0.07	0.06 ± 0.02	0.06 ± 0.02	----
Humulene Epoxide II			0.04 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	----
G-Eudesmol			----	0.23 ± 0.04	0.21 ± 0.04	0.24 ± 0.08	337.69 ± 16.30
2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol	1520		----	0.24 ± 0.13	0.19 ± 0.03	0.24 ± 0.09	601.39 ± 26.71
Subtotal €			0.42 ± 0.30 ^B	1.03 ± 0.16 ^A	0.73 ± 0.12 ^{AB}	1.09 ± 0.21 ^A	1072.97 ± 48.76
Overall Total €			51.43 ± 14.77	29.35 ± 3.80 ^B	38.07 ± 8.45 ^{AB}	45.16 ± 2.84 ^A	5868.27 ± 52.98

N = 3; Means +/− STD, * N = 3;—indicates a compound was not detected. Subtotals were calculated for groups by totaling the sum of each run. Values bearing different letters are statistically significant. €, statistical differences ($p < 0.05$), ¥: statistical differences ($p < 0.10$). ^G: represents no statistical differences at either (0.05 or 0.1). * Odor descriptors were obtained from the following databases: ASBC Sensory [18], FlavorNet [19], Serviss et al., 2024 [20], and PubChem Compound Summary [21].

Cannabis sativa and *Humulus lupulus* L. both contain elevated levels of terpenes or isoprenoids. These compounds make up to 3–5% of the dry mass of the female inflorescence [9,22]. This is the primary reason that the terpene groups were of interest for this experiment because of their high concentration in both hops and hemp [23], which explains why there are some terpene compounds found in the base beer. Mangum, a bittering hop, was utilized for this study. As previously described, it was added at the beginning of the boil, allowing it time to completely isomerize, providing antiseptic properties to the beer [24]. It also explains why tiny amounts of terpene-based compounds are found in the base beer despite not having any hemp added to it. Our results agree with Ascrizzi et al. (2020), in that adding hemp to the experimental (3, 6, and 9% ABV) beer saw an increase in the concentration of terpene compounds. Overall, there were statistically significant differences ($p < 0.05$) between the overall concentration of volatile compounds. Ethanol concentration has been shown to impact not only volatile compounds [25] but carbon dioxide solubility as well [26]. The use of inflorescences (flowers), leaves, and seeds of the industrial hemp plant are heavily regulated by the United States government when it comes to their use in beer. Any hemp plant material that will be ingested or made into medicine must be tested to ensure that it complies with the 2018 Agricultural Improvement Act. When it comes to designing recipes for alcoholic beverages, the Alcohol and Tobacco Tax and Trade Bureau (TTB) must approve the use of ingredients. Currently the only portion of industrial hemp approved for using by a brewer is hemp seeds. However prior to use, the seeds themselves must be submitted for analysis to ensure that there is no detectable trace of THC [8].

Extraction efficiency is known to increase based on ethanol concentration. Hops and hemp do share a number of volatile compounds. β -caryophyllene, α -humulene, D-Limonene, and myrcene are some of the most abundant terpenoids found in both cannabis and hops [9]. Monoterpenoid and sesquiterpene groups showed that ethanol concentration did influence the extractability, based on the increase in the overall concentration of the total volatiles and their statistical differences.

It is well documented that *C. sativa* possesses its own exclusive aromatic profile, which is reliant upon cultivar [23]. Previous sensory analysis conducted by Mediavilla and Steinmann (1997) showed that cultivars of higher levels of monoterpenes were considered more desirable and had a more pleasant aroma [27]. In this study, monoterpenes made up approximately 42% of the total overall aromatic profile for the essential oil extracted from the hops, while it makes up approximately 15–18% of the overall total aromatic profile. Ascrizzi et al. (2020) measured the headspace of a hemp beer that utilized hemp in their whirlpool and found that monoterpenes, oxygenated terpenes, and sesquiterpenes made up approximately 15.4% of the overall aromatic profile [23]. In comparison, the terpene groups made up 21–27%, dependent upon ethanol concentration. Terpenes extracted during dry-hemping had a greater impact on the overall aromatic profile than higher ethanol concentration. Mediavilla and Steinmann [27] found that myrcene, α and β -pinene, limonene, and β -caryophyllene made up approximately 50–90% of the volatile composition of the essential oil from *C. sativa*, while our study more closely followed that of Pieracci et al. (2021) regarding the essential oil composition [28]. Myrcene, α and β -pinene, limonene, β -caryophyllene, and linalool composed approximately 74% of the total concentration for all the terpenes. Thus, further studies are needed to determine which volatile compounds would be extracted during dry-hemping. This is important if hemp is to be used as a potential aromatic substitute for hops.

Figure 1 shows a 2D-PCA plot for the total volatile compounds observed in this study. The addition of the hemp to the experimental beer is shown to have had an influence on the overall volatile profile due to the visualization of the clustering. Based on the experimental beers clustering together, it shows that ethanol does influence the aromatic profile of the experimental beers. It makes sense that, when comparing the concentration of certain chemical groups such as monoterpenes or sesquiterpenes, 6% would not be statistically different from 3 and/or 9% ABV, but 3% is statistically different from 9%. This can be

observed in the PCA plot. It should be noted that there was a wider than expected amount of variability within the 6% than the 3 and 9% ABV beer, which could be explained by the lack of statistical differences for some of the terpene groups. There is the appearance that the clusters of the 3% ethanol beers are separated from the 9% ethanol beers. Whole hemp flowers were used for this experiment, which could have impacted extraction efficiency. To improve efficiency, the whole flowers should have been coarsely ground to increase their surface area [8]. These data suggest that further investigation into the impact of the addition of hemp to beer.

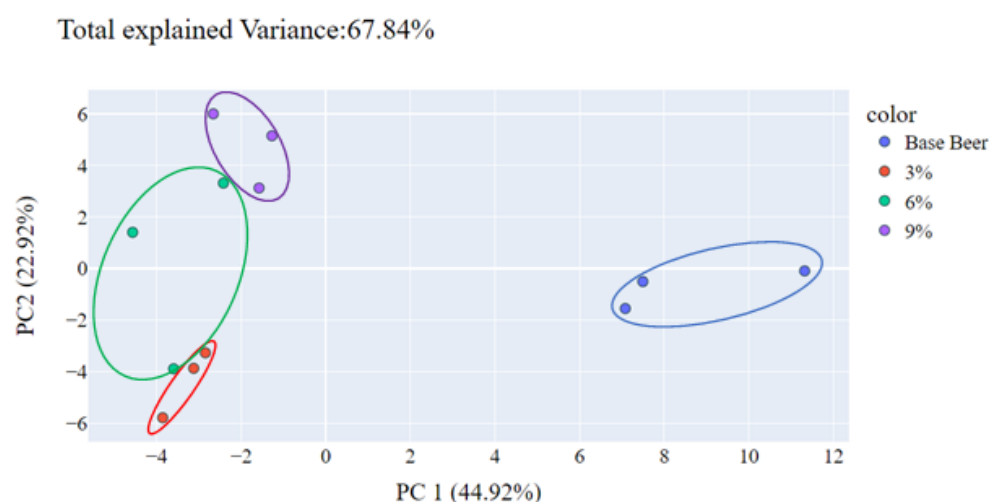


Figure 1. Principal component analysis (PCA) of the total volatile compounds; N = 3; base beer, 3% ethanol, 6% ethanol, and 9% ethanol beer.

4. Conclusions

These experiments were designed to investigate the impact of ethanol concentration on the inclusion of cannabinoids and the volatile compound composition following the dry-hemping of beer. Negligible impact was observed on the inclusion of the cannabinoids measured over the range of ethanol concentrations. Ethanol concentration was shown to impact the concentration of the volatile composition of the beer. The ethanol concentration was shown to have the greatest influence on the monoterpenes and sesquiterpenes and the overall concentration of the volatile organic compounds identified in dry-hemped beer. In addition, no statistical difference in the terpene group concentration was observed. This initial investigation shows the potential of using hemp as a flavor additive in beer. However, further work needs to be carried out using coarsely ground hemp instead of whole flowers to improve extraction efficiency and potentially allow for the most significant impact on composition.

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